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Preclinical mouse models for immunotherapeutic and non-immunotherapeutic drug development for pancreatic ductal adenocarcinoma

Mengni He[1], **MacKenzie Henderson**[2,3], **Stephen Muth**[2,3], **Adrian Murphy**[2,3,4], **Lei Zheng**[2,3,4]

[1]Department of Cell Biology, Baltimore, MD, USA

[2]Department of Oncology, Baltimore, MD, USA

[3]The Sidney Kimmel Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[4]The Precision Medicine Center of Excellence (PMCoE) Program for Pancreatic Cancer, Baltimore, MD, USA

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is in urgent need of better diagnostic and therapeutic methods due to its late diagnosis, limited treatment options and poor prognosis. Finding the right animal models to recapitulate the tumor molecular pathogenesis and tumor microenvironment (TME) complexity is critical for preclinical immunotherapeutic and non-immunotherapeutic treatment developments. In this review, we summarize and evaluate popular preclinical animal models including patient-derived xenograft models, humanized mouse models, genetically engineered mouse models, and syngeneic mouse models. Through comparisons between these models in different research settings, we hope to provide guidance in finding the most relevant preclinical models to suit various research purposes.

Correspondence to: Lei Zheng, MD, PhD. 1650 Orleans Street, CRB1 Room 351, Baltimore, MD 21287, USA. lzheng6@jhmi.edu.

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Keywords

Pancreatic Ductal Adenocarcinoma (PDAC); mouse models; xenograft; humanized mouse model; hemispleen

Molecular pathogenesis of pancreatic ductal adenocarcinoma (PDAC)

PDAC is the third leading cause of all cancer-related deaths. It has a 5-year survival rate of only 9% in the United States (1). The low survival rate is mainly due to early metastasis and limited therapeutic options. Only 10–20% of patients are diagnosed with a surgically resectable tumor due to delay in diagnosis with a 5-year survival rate of 37%, whereas 53% of patients are diagnosed at a metastatic stage with a 5-year survival rate at 3% (1,2). Common therapeutic options for PDAC patients include surgery, radiation therapy, and chemotherapy, which aim at relieving symptoms and/or extending survival. Surgery is the only possible cure, but it is only suitable for less than 20% of the patients (1,3). Hence, it is of vital importance to develop new diagnostic tools and therapeutic strategies, which rely heavily on the use of proper preclinical models.

In the past couple of decades, the mechanism for the pathogenesis of PDAC has been extensively studied. It is a common belief that mutations in *KRAS, CDKN2A, TP53, BRCA2* and *SMAD4* are important for PDAC tumorigenesis (3). The oncogenic mutation in *KRAS* is considered the first genetic change to initiate pancreatic intraepithelial neoplasia (PanIN) lesions and are found in nearly 90% of invasive PDAC tumors in patients (4). Mutational activation of KRAS results in induction of cell proliferation, cell survival, invasion, and stimulation of other oncogenic signaling pathways (5). Different KRAS mutations can occur within the same PanIN lesion and PDAC tumor, further supporting its role in PDAC progression. The loss of the tumor suppressor gene *CDKN2A*, encoding for p16 (INK4A), is another common mutation found in 90% of the PDAC tumors (6). The gene *CDKN2A* encodes cyclin-dependent kinase (CDK) inhibitor which inhibits cell cycle progression. Hence, the loss of *CDKN2A* function accelerates PDAC progression (7). Loss of tumor suppressor gene *TP53* function via missense alteration of the DNA-binding domain occurs in more than 70% of PDAC patients, contributing to genomic instability and telomere dysfunction during cancer progression (8,9). Genetic alterations resulting in loss of SMAD4 function occur in more than 50% of PDAC patients. The SMAD4 protein is critical for transforming growth factor beta (TGF-β) signaling. Loss of SMAD4 function abrogates the SMAD4-dependent TGF-β pathway, promoting cancer cell growth (10). Decrease expression of PTEN has also been found in 70% of PDAC patients, suggesting its role as a major tumor suppressor in PDAC (11). The reduced level of PTEN is associated with an enhanced PI3K/Akt signaling, promoting PDAC metastasis (12).

Due to the importance of these common genetic alterations in PDAC pathogenesis, transgenic mouse models of PDAC have been developed to recapitulate these genetic alterations and study their specific role(s) in the molecular pathogenesis of PDAC.

In addition to genetic mutations, tumor-stroma interactions within the heterotypic microenvironment also greatly contribute to the pathogenesis of PDAC (3). The majority of

PDAC tumors are predominately composed of fibroblasts, endothelial cells, extracellular matrix (ECM), hematopoietic cells, and myeloid cells (13). Deposition of ECM components and proliferation of stromal fibroblasts are often found within the tumor microenvironment (TME), contributing to the complexity of the TME in PDAC. Pancreatic stellate cells (PSCs) are another major component within the TME and once activated, they transition to myofibroblasts to secret ECM proteins (14,15). Fibroblasts can also negatively impact the immune cell infiltration in the TME by secreting CXCL12 to prevent the entering of CXCR4+ T cells into the TME (16). Secreting a panel of chemokines with heterogeneous functionalities including CCL5, CCL2, CCL17, IL-1, IL-4, IL-13, and IL-23, fibroblasts can also hinder macrophages and T cell functions (15-17). A strong immunosuppressive microenvironment is a renowned characteristic of PDAC, achieved by a high number of myeloid cells including myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) (13). Coupled with the relative absence of T cells within the TME, the poor response rates of chemotherapy, targeted therapy, and immunotherapy in treating PDAC is attributed to the desmoplastic immunosuppressive TME. Therefore, it is essential for a preclinical animal model to recapitulate the TME of human PDAC.

Thus, an ideal preclinical model of PDAC needs to represent both molecular pathogenesis and the TME of human PDAC. In this review, we discuss the advantages and disadvantages of key animal models that are currently used in preclinical research (Tables 1,2).

Patient-derived xenograft models

Patient-derived PDAC models were one of the first preclinical models used for PDAC research. As early as 1963, researchers had utilized human PDAC cell lines derived from primary pancreatic tumors to characterize and test anti-cancer drugs (18). These stable immortal cell lines are relatively homogenous, easy to use, and cost-effective. Through proteomic and transcriptomic approaches, studies have identified key characteristics of PDAC cell lines including mutations in KRAS, p53, and SMAD4. Transcriptome analysis of PDAC cell lines has also revealed a list of oncogenic miRNAs regulating tumor promoting genes like *TP53, Bcl2, Rac1*, and *CD40* (19-22). Despite the convenience of patient derived cell lines, they are less than optimal for PDAC studies. Maintained in culture, highly mutative cancer cell lines may accumulate genetic changes over multiple passages, and thus may generate new characteristics to impact the reproducibility of related experiments. In addition, different PDAC cell lines can cause differences in research outcomes, failing bench-to-bedside transition (23,24). The limited variety of human PDAC cell lines can only represent a limited patient population (25). Substantial differences in protein expressions exist between cell lines and tumors in patients. In addition, the PDAC cell line maintained as monolayer culture may be selected for subpopulations with additional mutations that result in growth advantages (26,27). Therefore, direct patient-derived tumor tissue specimens are utilized to minimize the above-described disadvantages of the established monolayer cultured cell lines. They are subcutaneously implanted (under the skin) in the immunocompromised mice and passaged from one mouse to another. Pieces of dissected tumors can be cryopreserved for long-term storage. However, the success rate of direct tumor tissue implantation is dependent on the aggressiveness of the cell lines or the resected tissues. Thus, the growth of implanted tumors is correlated with poor prognosis and more

aggressive tumors in patients (4,6). However, using direct tumor tissue implantation also provides the potential for personalized medicines. By either injecting tumor cells or tumor pieces derived from tumor excision or biopsy, mice with those tumors harbor intratumoral heterogeneities as the patient (28). Researchers and physicians can then better predict the outcome of a treatment by testing drugs and therapeutic methods on those mice. Yet, generating a patient-derived model from primary tissues can take up to eight months, making it challenging for routine diagnostic use in a clinical setting given the short survival time of PDAC patients.

Commonly used xenograft models include cell line-derived xenograft (CDX) or xenograft (PDX) model by introducing human PDAC cell lines or PDAC tumor tissues into immunocompromised mice respectively (29-31). The discoveries of T cell deficient nude athymic mice, as well as B and T cell deficient severe combined immunodeficient (SCID) mice, allowed researchers to overcome the species barrier to develop xenograft models using human specimens (32,33). Stable human pancreatic cell lines or resected tumor tissues can be injected or transplanted into a mouse subcutaneously (34). Such an approach is often favored for non-immunotherapeutic drug screening as it is relatively cost-effective and convenient (35). The implanted tumor is easy to visualize and easy to measure for determining drug efficacy. Depending on the aggressiveness and invasiveness of the cell lines, tumors can be palpable within 2-6 weeks (36). However, the biological relevance of subcutaneous models is limited as PDAC patients often develop metastasis, yet subcutaneous murine models often do not. In addition, drug delivery and tissue penetration in human patients would not be recapitulated in the subcutaneous models of PDXs and CDXs.

Compared to subcutaneous models, orthotopic xenograph models, implating PDAC cells or resected tissues into the pancreas of nude mice or SCID mice, allow a better resemblance of human PDAC (37,38). Although the procedure requires higher surgical techniques and is more costly, it has a higher predictive value to generate more biologically relevant data (29). Orthotopic models often show stable growth kinetics, molecular diversities, and measurable metastasis, allowing for better identification of tumor genotypes and morphologies, as well as non-immunotherapeutic drug testing (39,40). However, continuous monitoring of tumor growth is challenging in orthotopic. The current common method is through ultrasonography. Nude and SCID murine models are more susceptible to infections and other health problems with their compromised immune system, potentially obstructing experiments. In addition, during the generation of PDX, a subpopulation of tumor cells with stronger proliferative advantages is likely to outgrow the others, resulting in an inevitable selection of more aggressive cancers in xenograft models, and therefore limiting the researchable targets and cancer genotypes (41-43). As immunocompromised murine models lack heterogeneous stroma and an intact immune system, they are not ideal for the development of drugs targeting TME and the immune system.

Humanized mouse model

To compensate for the immunodeficiency of SCID or nude mice while maintaining the clinical relevance of using patient-derived tumor cells and tissues, humanized mice bearing

mutations in the IL2 receptor common gamma chain (*IL2rg*^{null}) in the non-obese diabetic (NOD)/SCID background were developed (44-46). With less NK cell activity from NOD background and the severely impaired B and T cell functions from SCID background, these mice support engraftment with human tissue, peripheral-blood mononuclear cells (PBMCs) and hematopoietic stem cells (HSCs), enabling the modeling of human immunity in immunocompromised mice (47,48). PBMCs allow the introduction of mature human leukocytes, especially activated T cells, whereas HSCs can potentially introduce all human hematopoietic lineages (49). Three commonly used humanized mice strains are: NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl} (NSG), C;l29S4-*Rag2*^{tm1Flv}*Il2rg*^{tm1Flv} (BRG), and NODShi. Cg-Prkdc^{scid}I12rg^{tm1Sug} (NOG) (47,49). While NOG mice have truncated IL2 receptor common gamma chain, NSG and BRG mice have complete null allele of the gamma chain.

Under this unique genetic background, patient-derived PDAC tissues or cell lines can be transplanted into these mice while potentially maintaining the tumor and TME heterogeneities. In general, compared to patient-derived cell lines, PDXs are better for therapeutic screening with higher correlation of clinical efficacy (49). Recently, a ‘AVATAR’ approach had also been taken to use humanized mice for personalized medicine to test for the efficacy of treatments (50).

Engraftments of human immune components enable characterization of interactions between the tumor and the immune systems, as well as providing valuable insights for both cell-based and antibody-based immunotherapy development (51). For instance, genetically modified T cells with chimeric antigen receptors (CAR-T cells) have been introduced into NSG mice to investigate the anti-tumor potentials of immunotherapies (52,53). However, CAR-T therapy showed strong adverse events in patients but not in humanized mice, possibly due to the lack of human targets in normal mouse tissues. Another popular approach using humanized models for PDAC cellular immunotherapy is called adoptive NK cell therapy aiming at stimulating the anti-tumor activity of NK and NKT cells (54). Humanized positive and negative immunological regulators and ligands of interests have also been knock-in to immunocompromised mice, including PD-L1, CD47, BTLA, OX40, etc., providing valuable research tools for studies of clinical candidates, especially combination therapies targeting immune-oncology checkpoints. Humanized mice also allow the studies of human antibody-dependent cellular cytotoxicity (ADCC).

Although humanized mouse models allow investigations of novel immunotherapies, these animals do not harbor the full human immune system. The remnant mouse innate immunity in humanized mice results in limited lymph node development, HLA incompatibility between engrafted human immune system and implanted PDX, and an inability to mimic human immune cell trafficking, all of which are major shortcomings in the currently used humanized mouse models (55). Pancreatic cancers are traditionally classified as non-immunogenic (“cold”) tumors due to its lack of T cell infiltrations. Such property of human PDAC partially explains the negative outcome of immune checkpoint inhibitors in clinical settings (56). Yet, in current PDAC research, tumor implantations can cause T cell infiltrations into the tumors due to histoincompatibility, subsequently changing these non-immunogenic “cold” tumors into artificially immunogenic “hot” tumors. Therefore, introducing human tumors into immunocompromised mice can still be recognized as foreign

substances, causing T cell infiltration in the TME and leading to false results in immunotherapeutic studies (56,57).

Genetically engineered mouse model (GEMM)

Although immunocompromised mouse models using human cells and tissues allow good representation of human disease, immunocompetent mouse models of PDACs are still the mainstream of preclinical mouse models. KRAS mutations occur in more than 90% of PDAC patients (58). In addition, endogenously expressing $Kras^{G12D}$ allows the initiation of PanIN, which can spontaneously progress into aggressive and metastatic diseases. Taken the abundance and pathogenic significance of KRAS mutation, researchers had started generating genetically engineered mouse models (GEMMs) harboring KRAS mutations. Although KRAS mutation alone is not sufficient to induce PDAC, in combination with other common PDAC mutations like INK4A, TP53, SMAD4, and TGF-β, various GEMMs had been developed on the base of $Kras^{G12D}$ mutations (59,60) (Table 3).

Previous studies had identified a series of important transcription factors during the development of the normal pancreas including early developmental homeodomain-containing transcription factors MNX1, NKX6-1, and PDX1, as well as basic helix-loop-helix transcription factor p48 (67,68). In mice, dorsal and ventral prepancreatic regions are formed independently at around embryonic day E8.5, followed immediately by the appearance of epithelial buds at E9.0 (69). Under the expressions of sonic hedgehog (SHH), retinoic acid (RA), fibroblast growth factor (FGF), and bone morphogenetic protein (BMP), the dorsal and ventral prepancreatic regions are specified (70). To further establish the pancreatic identity during the embryonic stage,PDX1 is expressed to induce the morphogenesis of pancreatic epithelium and pancreatic endocrine cell differentiation (71). The importance of PDX1 during pancreatic development was shown in PDX1-deficient mice as they develop only the pancreas buds, but not functional pancreas (71,72). P48, bound to transcription factor PTF1, is essential for exocrine cell differentiation and proliferation (73). The inactivation of PTF1-p48 changes the fate of pancreatic progenitor cells to duodenal epithelium cells (74). Due to the importance of PDX1, *LSL-*$Kras^{G12D}$; *Pdx1-Cre* mice have subsequently been engineered and used for pancreatic cancer studies. In short, a *Kras* mutation commonly found in PDAC patients was generated on exon 1 by changing a G to a D, V or R at codon 12 (61). A vector containing the genetic material that inhibits transcription generated with two functional LoxP sites flanking the genetic elements was then inserted into the upstream of the mouse genomic *Kras* locus (58). The *LSL-*$Kras^{G12D}$ mice were then interbreed with *Pdx1-Cre* mice, where the Cre recombinase was expressed only in pancreas (Figure 1A). Through a series of excision-recombination events, the *LSL-*$Kras^{G12D}$; *Pdx1-Cre* animals only express mutant KRAS in the pancreas. Such endogenous expression of KRAS mutant initiates PanINs when the animals are as young as two weeks old. As the animals age, higher-grade of PanINs occur and with higher frequencies.

Similarly, a more robust transgenic mouse model was generated by introducing *LSL-*$Trp53^{R172H}$ into *LSL-*$Kras^{G12D}$ animals and then interbreed *LSL-*$Kras^{G12D}$*; LSL-*$Trp53^{R172H}$*; Pdx1-Cre* mice with *Pdx1-Cre* mice (75) (Figure 1B). The resulting LSL-KrasG12D; LSL-Trp53R173H; Pdx1-Cre (KPC) triple mutant animals develop spontaneous

PDAC with cachexia, abdominal distension, bowel and biliary obstruction, corresponding to the typical clinical findings in PDAC patients. PDAC progression in KPC mice also closely resemble the human disease as they develop PanIN by the age of 8 to 10 weeks, and invasive tumors by the age of 16 weeks (63). As the disease progresses, the tumor will metastasize to lung, liver, diaphragm, and adrenals in these animals, mirroring human PDAC metastasis. KC-*Brca* mice have also been engineered harboring mutations in tumor suppressor genes *Brca2* and *Kras*, showing that BRCA2 mutation promotes KRAS-driven pancreas carcinogenesis (62). BRCA2 mutation has also been introduced into KPC mice, providing a more clinically relevant model for PDAC research (62,76). The KC-*Brca* mice and KPCB mice develop pancreatic tumors in two to three weeks but with a shorter survival of approximately 4 to 5 weeks. Similarly, since the inactivation of G1 cyclin-kinase inhibitor $p16^{INK4A}$ is found in majority of PDAC patients, mice with loss-of-function p16 in combination of KRAS mutation develop highly invasive tumor and died by the age of 11 weeks (60). SMAD4 mutation has also been introduced into KC mice. Mice with SMAD4 mutations and KRAS mutation exhibit early rapid development of intraductal papillary mucinous neoplasia (IPMN), yet failed to develop aggressive pancreatic malignancies (63). Mice with TGF-β knockout and $Kras^{G12D}$ mutant driven by *Ptf1a*-Cre-LoxP system were also generated as 55% of PDAC patients have TGF-β mutation. TGF-β knockout promotes KRAS-driven tumor by transforming PanINs into PDAC tumors. KC-*Tgfb2* mice develops tumors at the age of 6–7 weeks with a medium survival of 8.4 weeks (64). Recently, Talbert *et al.* has developed a $LSL\text{-}Kras^{G12D/+}$; $Ptf1a^{ER\text{-}Cre/+}$; $Pten^{f/f}$ (KPP) model to recapitulate cancer-induced cachexia (66). Cachexia is a common cancer-induced syndrome characterized by skeletal muscle loss, relating to increased morbidity and mortality (77). PDAC patients often meet the criteria of cachexia upon diagnosis. However, there is no good treatment targeting cancer-induced cachexia, partially due to the lack of an appropriate animal model. Previously, the KPC model was often used for cachexia-associated research as they exhibit cachexia syndromes. However, Talbert *et al.* has demonstrated that the cachexic phenotypes of KPC are different from the cachexic phenotypes in PDAC patients where their novel KPP model can better represent the progressive wasting phenotype in human PDAC.

As *Pdx1*- or *Ptf1a/p48*-driven Cre-LoxP systems allow expression of $Kras^{G12D}$ mutant starting in early pancreatic development and in all epithelial progenitor cells, it is hard to pinpoint the cell-of-origin of PanINs and PDAC tumors (78). In addition, human PanINs and PDAC tumor rarely initiate during pancreatic development. To address this issue, temporal KRAS mutant mice can be generated by crossing mice with conditional endogenous $Kras^{G12V}$ oncogenes in acinar pancreatic cells with bitransgenic *Elas-tTA/tetO-Cre* mice that can express an Elastase promoter controlling Cre recombinase in a tet-off system (65). Using X-gal staining, β-galactosidase activity served as a marker for the expression of KRAS mutant under stimulation, proving the selective expression of $Kras^{G12V}$ in acinar and centroacinar pancreatic cells, inducing PanINs and invasive PDAC. An inducible system can also be applied to studies of other oncogenes and tumor suppressor genes. For instance, in combination of a Cre-LoxP system and a Flp-FRT system, YAP expression can be switched off in the background of spontaneous KRAS mutated pancreatic tumors in immunocompetent mice (79). By incorporating fluorescence tags to the YAP protein, the

expression pattern of YAP and its effect in PDAC development was revealed. In addition, in the settings of understanding epithelial-to-mesenchymal transitions of pancreatic cells, a yellow fluorescence protein (YFP) protein can be introduced into KPC mice by crossing KPC mice with *Pdx1-Cre; RosaYFP* mice (80). Using this GEMM, researchers were able to trace YFP tagged mutated pancreatic cells migration even before signs of PDAC tumors. GEMMs provide an opportunity to study not only the establishment, but also the progression of tumors. They reveal valuable information for cancer metabolism and cancer prevention, especially on delaying the precursor lesion progression and preventing both local and metastatic diseases.

As transgenic mouse models recapitulate the tumorigenesis of PDAC, they are useful tools for novel biomarker discoveries in early stages like PanIN as well as in the metastatic stage. Using a proteomics approach, a recent study found an enrichment of a cell surface proteoglycan, glypican-1 (GPC-1) in cancer cell-derived exosomes using the KPC model (81). Levels of GPC+ circulating exosomes correlate with tumor burden and survival in both patients and KPC mice, providing a potential non-invasive diagnostic biomarker for early PDAC detection (82). Other molecules important for PDAC development and metastasis including USP9X (83), Annexin A2 (84), and cytokine tissue inhibitor of matrix metalloproteinases 1 (TIMP1) (85) were also characterized using GEMMs. In addition to the biomarker discoveries, these mouse models also allow developments of novel therapeutic strategies. Preclinically evaluated in KPC mice, pegylated recombinant human hyaluronidase (PEGPH20) was developed to degrade hyaluronic acid (HA) in the ECM, enhancing the drug delivery efficiency (86). Characterization and preclinical study of IPI-926 in KPC mice, an inhibitor of hedgehog signaling pathways in KPC, also lead to the development of a series of hedgehog signaling protein inhibitors and combinational therapeutic strategies (87-89). Unfortunately, human clinical trials of IPI-926 plus gemcitabine or FOLFIRINOX were terminated early due to severe detrimental effects (90). Focal adhesion kinase (FAK) inhibitors had previously found to reduce tumor progression and are recently studied in KPC mouse models in combination with immune checkpoint inhibitors (91).

GEMMs are also largely used for PDAC immunobiology studies as well as PDAC immunotherapy developments. Since PDAC lesion arises spontaneously in the GEMMs, especially the KPC mouse model, it reproduces the leukocyte complexity and the immune cell infiltration in the TME as observed in human PDAC patients (16,63,92,93). For instance, strong infiltration of F4/80+ macrophages and low levels of effector T cells were found in the primary lesion in both KPC mice and PDAC patients (64,94). Preclinical trials or “co-clinical” trials of immunotherapies have been utilizing these GEMMs. Simultaneously conducting a human trial and mouse studies revealed that a CD40 agonist can recruit circulating monocytes to exhibit anti-tumor and anti-fibrotic effects, causing tumor progression in both KPC mice and humans (95,96). KPC models have also enabled studies to limit T cell infiltration into the tumor tissue including CXCL12 leading to a clinical trial of a CXCR4 inhibitor therapy in combination with anti-PD-L1 antibody (16).

In short, GEMMs represents the whole tumorigenesis of PDAC in a mammalian system, allowing researches for cancer preventions and metastasis prevention. While GEMMs are

extensively used in current PDAC research, they are expensive and time consuming. In addition, it is hard to isolate and characterize the tumor from the animal due to the lack of neoplastic cellularity. The most common method for tumor growth measurement and monitoring using ultrasonography is time consuming and labor intensive (64,97). Due to the challenges of monitoring tumor sizes throughout the experiments, the outcomes of therapeutic testing using GEMMs are often evaluated by survivals. In addition, individual difference of tumorigenesis between mice often complicate the results of experiments. Intrinsic differences between rodent and human proteins also diminish the predictive values of GEMMs in preclinical research.

Syngeneic mouse models

As the therapeutic role of the immune system has been increasingly recognized, it is pivotal to have a mouse model with a competent immune system. Syngeneic mouse models, developed by introducing genetically similar or identical, or immunologically compatible tumor cells or tumor tissues into immunocompetent mice orthotopically or subcutaneously, are good models for such studies. One of the earliest murine PDAC cell lines, Panc02, was established from chemically induced PDAC mouse models in 1984 (98). However, the genotype and phenotype of Panc02 fail to recapitulate the human PDAC disease as it does not harbor KRAS, p53, and PDX1 mutations (99). A good alternative is the KPC cell lines established from genetically engineered KPC mouse for their representative genetic mutations (100). However, recent reports showed that KPC cell lines are poorly immunogenic due to their similar growth rates in immunodeficient mice and in immunocompetent mice (101). This characteristic of the KPC cell line makes it an ideal model to recapitulate the “cold” TME of human PDAC. The orthotopic model can also more faithfully recapitulate the TME of human PDAC than the subcutaneous model. Another advantage of the orthotopic implantation model is to allow the control of the size of the implanted tumor pieces, simply by carefully cutting the tumor into pieces using a ruler, allowing for the comparison among multiple treatment groups and different combination therapies.

Syngeneic mouse models can be established in immunocompetent mice orthotopically into the pancreas, or subcutaneously under the skin. Transplantation of mouse-derived tumor tissues or injection of mouse-derived tumor cell lines allow the development of tumor and subsequently the intervention on the tumor under a competent immune system. Since the subcutaneously implanted tumor is not growing in its native organs, the biological relevance of subcutaneous models is limited compared to the orthotopic models. Syngeneic orthotopic models are also better at representing the cell-to-immune system interactions. However, recent reports showed that the tumor infiltration B cells in syngeneic orthotopic models are significantly less than in genetically engineered KPC mice, indicating that two models are still different and therefore should be used together for immunotherapeutic development (102). For instance, using a combination of GEMMs and orthotopic models, Foley *et al.* revealed the metastatic mechanism of PDAC characterized by SEMA3D autocrine signaling (103). Similarly, the preclinical efficacy of GM-SCF-secreting allogenic whole pancreatic tumor cell vaccine (GVAX) targeting Annexin A2 (ANXA2) was evaluated (104). It should be noted that tumor measurement in both GEMMs and orthotopic models can be challenging

and time-consuming. Nevertheless, some groups have developed expertise in using small animal ultrasonography to measure the tumors growing on the internal organs. In addition, luciferase can be genetically engineered into the cancer cell lines, enabling a more convenient tumor imaging measuring the intensity of bioluminescence (Figure 2).

Intravenous, intraperitoneal, and intrasplenic administration of mouse-derived cell lines also provides the capabilities to study lung metastasis, peritoneal and lymph node metastasis, and hepatic metastasis respectively (64,105,106). Since the tumor is established at secondary locations instead of its native organs, these models better represent the formation and characteristics of metastasized tumors after surgical resection. As most cases of PDAC were diagnosed at late stage and complicated by distal metastasis, preclinical models of metastases are particularly valuable for developing therapeutics that can target both primary tumors and metastases. Subcutaneous models rarely metastasize. The timing and progression of metastasis in orthotopic models and GEMMs largely vary and thus are not feasible for drug development purposes. Therefore, intravenous, intraperitoneal, and intrasplenic models are commonly used for lung, peritoneal and lymph node, and liver metastasis studies respectively.

In 2014, Soares *et al.* have developed an intrasplenic model to better study liver metastasis without the presence of primary pancreatic tumor. In short, the hemisplenectomy procedure is achieved by dividing the spleen in half followed by the injection of Panc02 or KPC tumor cells into a hemispleen with splenic blood vessels connecting to the liver (Figure 3) (105). The tumor cells will then travel to and seed in the liver through the splenic blood vessel. To prevent the peritoneal drop metastasis, the injected half of the spleen is removed, while the remaining half of the spleen continues to perform immunological and biological functions (64).

This model was first developed using Panc02 cells, a highly aggressive and tumorigenic, chemically induced mouse cell line in C57BL/6 mice (98). Therefore, despite the long history and widespread usage of Panc02 cell line, Panc02 cells lack clinical significance as it does not harbor representative mutations as in the human disease (100). Compared to Panc02 cell lines, KPC cell lines and other KC-derived cell lines are better representations of the human PDAC disease as they share more genetic mutations. If left untreated, mice with hemispleen tumor would die within a short period of time, typically 30–60 days. Yet, using Panc02 cell lines, the efficacy of generating tumor in mouse liver is nearly 100%. Liver metastasis burdens are similar among different mice. In addition, the tumor generated by hemispleen models can be easily accessed throughout therapeutic treatments using ultrasound and/or luciferase-expressing KPC cells (Figure 3B,C). In addition, survival is often used as an endpoint for the preclinical studies of experimental therapeutics. If left untreated, mice with hemispleen tumor will die within a short period of time, typically 30–60 days. In the background of immunocompetent mice, these models allow the discovery and preclinical development of novel immunotherapies or combination immunotherapeutics (107).

Conclusions

Different preclinical mouse models provide valuable information on different aspects of PDAC tumor development and therapeutic targets (Table 2). In summary, patient-derived xenograft models allowed close representation of the human disease counterpart by using human specimens while lacking the ability to represent early PDAC development stages, tumor-immune system interaction, as well as the potential for large-scale or high-through drug screening. Humanized mouse models, as alternatives of patient-derived xenograft models, allow the studies of immunotherapeutic targets. Genetically engineered mouse models recapitulate the tumor progression from early PanIN to metastasis and are ideal for most research purposes, but requires labor-intensive and time-consuming efforts. Syngeneic mouse models are efficient for both non-immunotherapeutic and immunotherapeutic studies targeting both primary and/or secondary tumors. It is therefore important to understand the advantages and disadvantages of each model system (Table 1). Multiple tumor models should be combined in a complementary way for each scientific research and drug development project.

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Figure 1.

Summary of the Generation of Selected GEMMs. (A) Generation of KP mouse by crossing *LSL-Kras*$^{G12D/+}$ mouse with *Pdx1-Cre* mouse; (B) Generation of KPC mouse by first crossing *LSL-Kras*$^{G12D/+}$ mouse with *LSL-Trp53*$^{R172H/+}$ mouse, then cross their offspring with *Pdx1-Cre* mouse. GEMMS, genetically engineered mouse models.

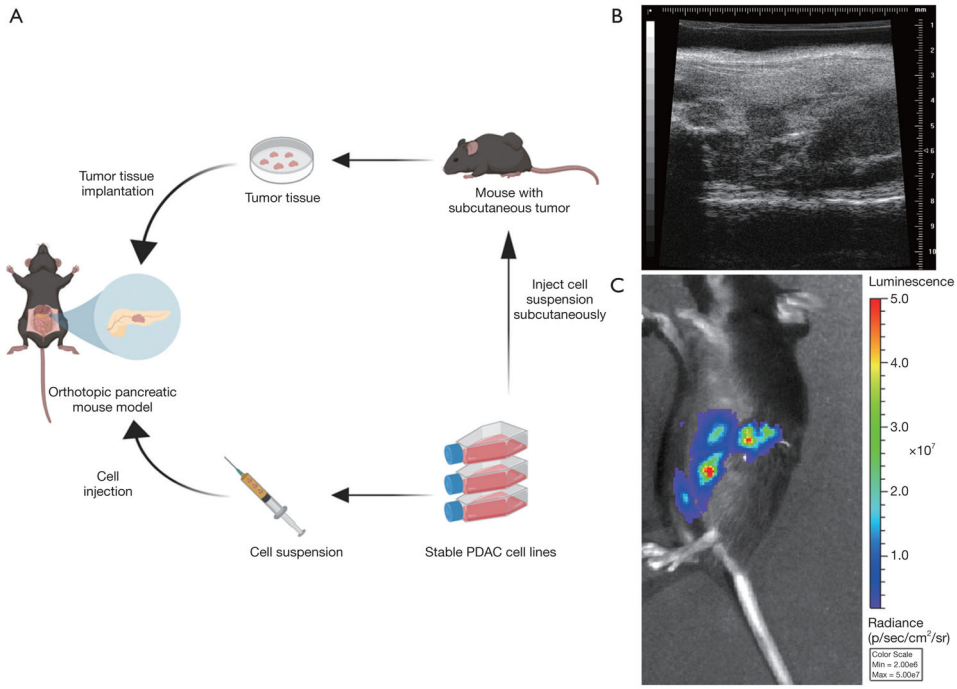

Figure 2.

Summary of orthotopic models. (A) Experimental Scheme of orthotopic pancreatic mouse model; (B) ultrasound imaging of PDAC in the orthotopic model; (C) IVIS Imaging of PDAC generated with luciferase-containing KPC cells in the orthotopic model. PDAC, pancreatic ductal adenocarcinoma.

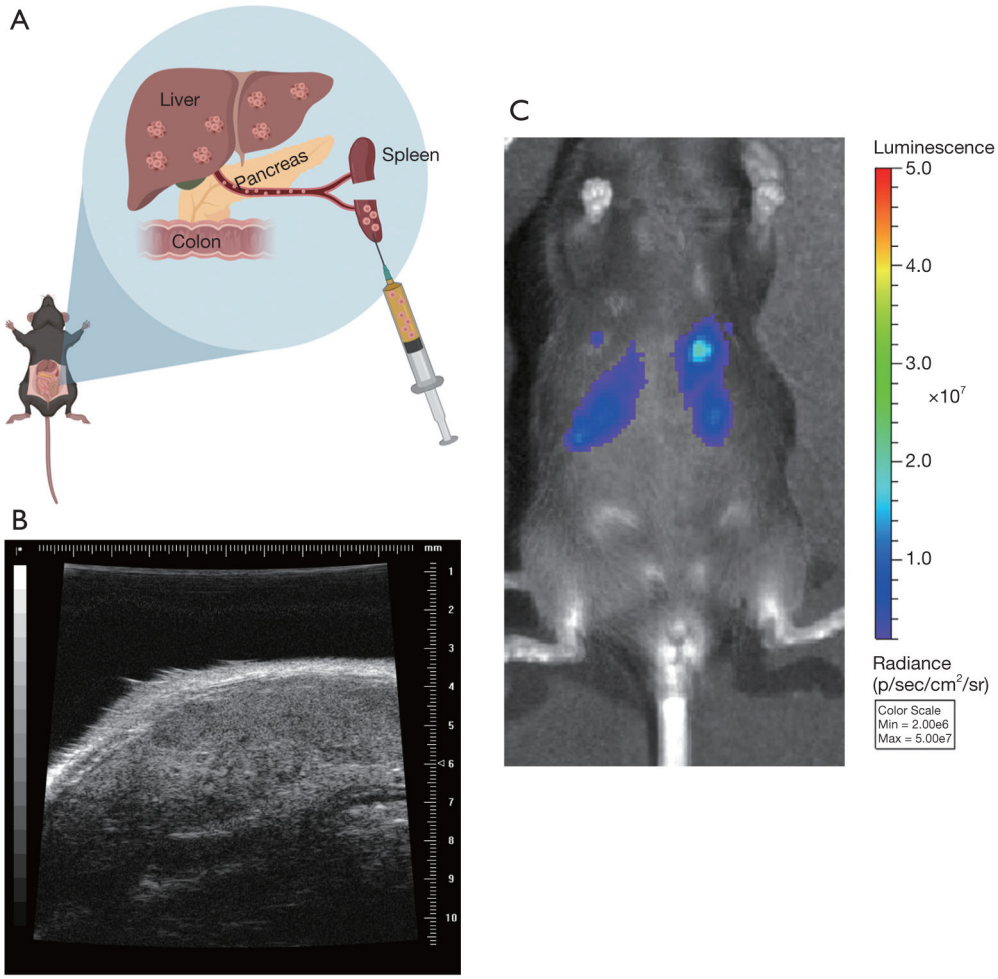

Figure 3.

Summary of hemispleen models. (A) Experimental Scheme of hemispleen mouse model; (B) ultrasound imaging of PDAC in the hemispleen model; (C) IVIS Imaging of PDAC generated with luciferase-containing KPC cells in the hemispleen model. PDAC, pancreatic ductal adenocarcinoma.

Table 1

Relative characteristics among different mouse models

Characteristics	Relative levels
Skills/facility/maintenance required	Cell lines < patient-derived mouse models < syngeneic mouse models = humanized mouse models < GEMMs
Easy to manipulate genetic background	GEMMs < syngeneic mouse models = patient-derived mouse models = humanized mouse models < cell lines
Time consumption	Cell lines < syngeneic mouse models = GEMMS = patient- derived models = humanized mouse models
Clinical relevance	Cell lines < syngeneic models < GEMMS < patient-derived models = humanized mouse models

GEMMS, genetically engineered mouse models.

Table 2

Types of research suitable using different model

	Early stage PDAC	Immune system	Tumor microenvironment	Metastasis	Large-scale and/or high- throughput drug screening
Cell lines (mouse-derived & patient derived)	No	Limited	No	No	Yes
Patient derived xenograft models—SQ	No	Limited	No	No	No
Patient derived xenograft models—orthotopic	No	Yes	Yes	Limited	No
Humanized mouse	No	Yes	Yes	Yes	No
Genetically engineered mouse model	Yes	Yes	Yes	Yes	No
Syngeneic mouse model—subcutaneous	No	Yes	No	No	No
Syngeneic mouse model—orthotopic	No	Yes	Yes	Limited	No
Syngeneic mouse model—hemispleen	No	Yes	Yes	Yes	No

PDAC, pancreatic ductal adenocarcinoma.

Table 3

Summary of GEMMs and Their Key Characteristics

GEMMs	Genetic mutation(s)	Time of mutant expression	Average time of tumor formation	Severity of PDAC development	Median survival	Reference
KP Mice with *Pdx1- Cre*	*LSL-Kras^{G12D}; Pdx1-Cre*	E8.5	6.25 months	From PanINs to aggressive and invasive PDAC in an age-dependent manner	1.5 years	(58)
KP Mice with *P48$^{+/Cre}$*	*LSL-Kras^{G12D}; P48$^{+/Cre}$*	E9.5	8.25 months	From PanINs to aggressive and invasive PDAC in an age-dependent manner	1.5 years	(58)
KPC Mice	*LSL-Kras^{G12D}; LSL-Trp53^{R173H}; Pdx1-Cre*	E8.5	2 to 3 months	From PanINs to aggressive and invasive PDAC in an age-dependent manner	5 months	(61)
KPC-*Brca* Mice	*LSL-Kras^{G12D}; LSL-Trp^{53R270H}; Pdx1-Cre; Brca2^{Tr}*	E8.5	1.5 months	Aggressive and invasive PDAC in an age-dependent manner	2.8 months	(62)
KC-Ink4a/Arf Mice	*LSL-Kras^{G12D}; Pdx1-Cre; Ink4a/Arf$^{lox/lox}$*	E8.5	1.25 months	Primarily locally invasive tumor	2 months	(60)
KC-*Smad4* Mice	*LSL-Kras^{G12D}; Pdx1-Cre; Smad4$^{lox/lox}$*	E8.5	7 to 12 weeks	Moderate PDAC	8 to 24 weeks	(63)
KC-*Tgfb2* Mice	*LSL- Kras$^{G12D/+}$; Ptf1a$^{cre/+}$; Tgfbr2$^{flox/+}$*	E9.5	6 to 7 weeks	Aggressive an Invasive PDAC	8.4 weeks	(64)
Inducible *Kras^{G12V}* Mice	*Kras$^{+/LSLG12Vgeo}$; Elas-tTA/tetO-Cre*	Inducible	Inducible	Dependent on Kras Mutant Induction	Dependent on Kras mutant expression	(65)
KPP Mice	*LSL-Kras$^{G12D/+}$; Ptf1a$^{ER-Cre/+}$; Pten$^{f/f}$*	E9.5	Initiated with tamoxifen between 24 and 28 days	Moderate PDAC, but progressive cachexic phenotype	3.5 months	(66)

GEMMS, genetically engineered mouse models; PDAC, pancreatic ductal adenocarcinoma.