Generation and application of patient-derived xenograft models in pancreatic cancer research

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Abstract
Objective: Pancreatic ductal adenocarcinoma cancer (PDAC) is one of the leading causes of cancer-related death worldwide. Hence, the development of effective anti-PDAC therapies is urgently required. Patient-derived xenograft (PDX) models are useful models for developing anti-cancer therapies and screening drugs for precision medicine. This review aimed to provide an updated summary of using PDX models in PDAC.

Data sources: The author retrieved information from the PubMed database up to June 2019 using various combinations of search terms, including PDAC, pancreatic carcinoma, pancreatic cancer, patient-derived xenografts or PDX, and patient-derived tumor xenografts or PDTX.

Study selection: Original articles and review articles relevant to the review’s theme were selected.

Results: PDX models are better than cell line-derived xenograft and other models. PDX models consistently demonstrate retained tumor morphology and genetic stability, are beneficial in cancer research, could enhance drug discovery and oncologic mechanism development of PDAC, allow an improved understanding of human cancer cell biology, and help guide personalized treatment.

Conclusions: In this review, we outline the status and application of PDX models in both basic and pre-clinical pancreatic cancer researches. PDX model is one of the most appropriate pre-clinical tools that can improve the prognosis of patients with pancreatic cancer in the future.

Keywords: Cancer research; Pancreatic ductal adenocarcinoma; Patient-derived xenografts; Precision medicine

Introduction
Pancreatic cancer is a well-known devastating disease that is often difficult to detect in the early stage. Among all the digestive tract tumors, the 5-year survival rate of pancreatic ductal adenocarcinoma cancer (PDAC) is still the lowest.[1] Its mortality rate nearly equals to the incidence rate,[2] and the overall 5-year survival rate is approximately 6%.[3] Data have shown an overall reduction in cancer-related mortality, while that for PDAC has increased; the prediction showed a similar trend.[1-3]

The survival rate of patients with PDAC remains the lowest since the diagnosis of PDAC in the majority of cases is extremely late; hence, surgical excision is almost impossible.[6] Less than 20% of patients are eligible for curative resection as pancreatic cancer is usually detected at a late stage. A multiple center study showed that 18.4% of pancreatic cancer patients were diagnosed at stage I or II and 81.6% at stage III or IV.[7] The poor prognosis of pancreatic cancer is also associated with its genomic complexity and heterogeneity and the absence of efficient therapeutic options. Gemcitabine, one of the Food and Drug Administration-approved adjuvant chemotherapies, only extended the survival rate for a few months for less than 20% of the patients with advanced pancreatic cancer.[8] To improve the survival rate, more effective therapies, better understanding of the biological mechanisms of pancreatic cancer development, recurrent, and metastasis; and therapeutic resistance mechanisms are required. The major challenge for PDAC is the availability of pre-clinical models that mimic the physiologically relevant microenvironments for the patients.

Comparison Between Patient-derived Xenograft Models and Other Models
To solve the problems caused by PDAC, several pre-clinical models have been established in vitro or in vivo. These include pancreatic cancer cell lines (CCLs), organoid, cancer cellular models implanted in mice, genetically engineered mouse models (GEMMs), circulating tumor cell
To date, several pancreatic CCLs have been established for not only in vitro but also in vivo studies. Xenografts developed by growing cell lines subcutaneously in immune-deficient mice are the most commonly used in vivo model before PDXs, even up to the present. Although CCLs are derived from cancer patients, recapitulating the genomic event typically leads to neoplastic changes. Some genomic differences between the tissues of origin and cell lines have been documented. The genetic transformations that CCLs gained during in vitro operation cannot be recovered when cells grow in vivo. Cell lines start to differentiate from the primary cancer cells after a couple of passages in immune-deficient mice. The irreversible transition from epithelial to mesenchymal phenotype is also observed in vitro. Moreover, tumors from different patients are highly heterogeneous, but the xenograft models using CCL are insufficient to represent complex tumor heterogeneity. Moreover, a tumor is a mixture of heterogeneous cells including cancer cells, fibroblasts, vessel cells, and immune cells. All these cells interact to form the tumor microenvironment, which is related to the growth, metastasis, and recurrence of cancer cells. However, CCLs are insufficient to recapitulate the tumor microenvironment. CCLs could not reflect the patient’s drug response accurately; hence, CDX is not an appropriate strategy for personalized medicine applications.

Regarding GEMMs, several pancreatic cancer models have been developed, including models that engineered the mutations in KRAS plus deletions or mutations in P53, Pdx1-Flp, P16INK4a, MIST, and SMAD4. In these models, orthotopic pancreatic cancer is similar to that in humans, and the spectrum of pathological changes has been observed in human pancreatic cancer, from pancreatic intraepithelial neoplasia and invasive ductal carcinoma. Although genomic analyses and next-generation sequencing have been developed rapidly over the past decades and several mutations have been identified, it is limited to specific, pre-defined genetic mutations, particularly to pancreatic stromal cells. These models usually have incomplete penetrance, long latency periods, and variable metastases. GEMMs perform the same mutations in all pancreatic tumor cells and are homogeneous, while pancreatic cancer is highly heterogeneous. Hence, GEMMs poorly reflect the genetic diversity of human pancreatic cancer. Moreover, the high cost of GEMMs limits their clinical application.

The study of PDXs has been conducted for more than 30 years. These models assumed to faithfully conserve the patient’s genetic mutations, particularly the patient-derived orthotopic xenograft (PDXO) models. Recently, PDX models have been established in different tumors and areas including but not limited to the breast, pancreas, colorectum, prostate, ovary, kidney, and lung. These models broadly represent the heterogeneity and the molecular diversities of the original tumors. Currently, PDXs are considered important clinical models for evaluating in vivo efficacies, biomarkers, and therapeutic responses, even for mechanism research. Considering all the special properties of PDX models mentioned above, several pancreatic cancer PDXs are established as experimental models with extensive applications in PDAC drug development. For classical chemotherapeutic drugs, PDX models based on clinical specimens can predict 90% drug sensitivity (19/21) and 97% drug resistance.

**Generation of Pancreatic Cancer PDX Models**

The detailed process on how to generate the PDX models in mice from fresh primary or metastatic human cancer tissues has been described in previous papers [Figure 1]. Models generated by draining fluid from malignant ascites have been reported. Besides the traditional way, models could be established by using CTCs from a single 10-mL blood drawn. Most pancreatic cancer PDX models are established with a common procedure in summary, the primary or metastatic tumor tissues are obtained from surgery resection or biopsy, and subsequently, tumors are implanted into small pieces or single-cell suspensions, either alone or in combination with some fibroblasts or mesenchymal stem cells. There are two main types of PDX models, heterotopic (subcutaneous implantation) and orthotopic, defined by the location. Studies have indicated that orthotopic models could recapitulate human cancer more closely than heterotopic models, and by using the relevant site for tumor-host interactions, the development of metastases, ability to study site-specific dependence of therapy, organ-specific expression of genes, and clinical scenario can be replicated. On the contrary, studies have demonstrated that heterotopic xenograft models often do not have a significant effect on human diseases because the subcutaneous microenvironment is not relevant to that of the organ site of primary or metastatic disease and rarely forms metastases, suggesting that heterotopic tumor models are not predictive when used to test the therapeutic responses of anticancer drugs. Furthermore, the renal capsule implantation, which was designed to increase the engraftment success rate and recapitulate human cancer more closely, is also an option. Despite the advantages mentioned above, an orthotopic model is difficult to generate, and subcutaneous implantation has relatively higher success rate and is a simpler procedure than the renal capsule implantation. Thus, the most common site of implantation is still in the flank of mice (subcutaneous implantation). Determining the most suitable host mouse strain to generate PDX models is critical. The types of host mice mainly include the following: nude (no functional T cell), severe combined immunodeficiency (SCID, no functional T and B cell), non-obese diabetic (NOD)-SCID (no functional T and B cell, natural killer (NK) cell impaired, or no obesity and diabetes), and NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ, no functional T, B, and NK cell). Recently, NSG mice were more commonly used in developing PDX models of some specific cancer types.

The pancreatic cancer PDX models commonly use NOD-SCID (no obesity and diabetes).

Pancreatic cancer PDX models’ implantation rates are among the highest, ranging from 42.9% to 60%,
studies have been conducted to achieve higher engraftment rates and generate models that recapitulate human tumors better.\textsuperscript{[33]} Tumor size, metastatic patient lesions, lymphovascular invasion, lymph node metastasis, and worse recurrence-free and patient survival may be related to PDX model formation.\textsuperscript{[25,32,34,35]} To verify the tumor establishment of the tumor model, the tumors were monitored for at least 100 days and measured until they reach a volume of 1000 mm\textsuperscript{3}.

Advantages of PDX Models in Cancer Research

Compared to CDXs, which have many limitations mentioned above, PDXs are better pre-clinical models because they represent human cancer biology and patients’ response to treatment. Hence, PDXs are used for personalized treatment. With the increasing number of pre-clinical studies using PDX models, we hypothesize that these models preserve the main characteristics of the original cancer, and these characteristics could be preserved well in mouse-to-mouse engraft passages.

Studies showed that PDX models were very close to the original tumors in many aspects as follows: PDX models preserve several critical characteristics of the original tumors, such as cellular and histological features, stromal elements, and similar tumor microenvironment. Several methods have been used in studies accessing the feasibility and properties of PDXs. The easiest and most convenient method is pathological comparison. The researches using pathological comparison proved that neither histological differentiation nor stromal content differed significantly between mouse xenograft tumors and their respective patient tumors.\textsuperscript{[25,36]} It is clear that the histological features are similar between the two types of tumors. Gene expression profiling, immunohistochemistry (IHC), and ribonucleic acid (RNA) sequencing are also applicable methods. Gene expression profiling revealed a high degree of conservation of gene expression with correlation coefficients of 93% to 99% when individual patient tumors were compared to PDX tumors.\textsuperscript{[25]} IHC analysis confirmed that the xenograft tumor cells were from human original tissue.\textsuperscript{[36]} Zhang et al.\textsuperscript{[37]} used the IHC method to assess the expression of pancreatic ductal-specific marker pancreatic and duodenal homebox 1, pancreatic tumor marker cytokeratin 8, and epithelial-specific marker epithelial cell adhesion molecule using the IHC method and demonstrated that all the isolated cells were pancreatic ductal epithelial tumor cells. RNA sequencing could also be used to measure the expression of specific genes. Short tandem repeat analysis showed that the genetic signature of PDX tumor matched closely to the signature of the patient.\textsuperscript{[36]} Mutation analysis, exome sequencing, and whole-genome sequencing also showed a high degree of association between primary and xenografted carcinoma.\textsuperscript{[37,38]} Furthermore, mouse-to-mouse propagation does not substantially change the functional characteristics of...
the grafted tumor but preserves their susceptibility/resistance status. Both differentiation state and stromal content were preserved at early and late passages for the mouse tumor samples. Studies that compared the PDX models’ responses to drug treatments over different passages have shown stable response rates across generations, which further supported the phenotypic stability of these models. Furthermore, studies have compared pancreatic cancer PDX models to the CDX models. The gene expression profiling data suggested that models of commercially established high-passage cell lines differed significantly from fresh human pancreatic cancer specimens. This result emphasized that fresh human cancer specimens for PDX should be used over CDX models.

PDXs have almost unlimited human tumor resources over conventional pre-clinical models such as CDXs. Recently, only several cell lines of each cancer type have been validated for CDX model establishment, and tumorigenesis, diverse growth curves, and variations of planted tumor could be the main limitations. The following risk should not be overlooked for CDXs: the genomic features of the specific cancer cells have a chance of loss during in vitro culturing process, specifically for the cell line with lower proliferation rate. Both the problems would be significantly solved by PDXs by the large number of clinical specimens and the stable maintenance of histological and genomic characteristics derived from the cancer patients.

Applications of PDX Models in Pancreatic Cancer Research

Drug screening and biomarker development

The high failure rate of clinical trials is one of the biggest challenges in anti-tumor drug development, specifically for pancreatic cancer. Currently, 5-fluorouracil/folinic acid, irinotecan, and oxaliplatin is the standard chemotherapy strategy for advanced pancreatic cancer with the objective response rate of approximately 30%. The poor performance of conventional pre-clinical models in predicting new drug’s efficacy and therapeutic response is associated with these unsatisfactory clinical results. Several studies using PDX model in other cancers such as breast, renal cell, non-small cell lung cancer, and colorectal cancer and squamous cell carcinoma of the head and neck have shown that the drug response or resistance in PDX models was significantly associated with those observed in clinical settings.

PDX models were considered as potential screening models for the discovery of novel therapeutic agents [Figure 2]. Centromere protein E inhibitor GSK923295 is a promising anti-cancer drug, but its function in hepatocellular carcinoma (HCC) remain to be fully elucidated. Tang et al. used PDX models to describe anti-HCC activities of GSK923295. A study involved 32 pancreatic cancer patients demonstrating the consistency between the PDX models and patients, which were both treated with gemcitabine. The result showed that when PDX models failed to exert anti-tumor efficacy, the clinical result was also negative. This association was also observed in other researches of the pancreatic cancer agents. Based on these data, PDX models have now become an essential part of new anti-cancer drug discovery. To meet different clinical needs, more options for pancreatic cancer PDX models have been developed. Hall et al. demonstrated the single-drug activity of oxaliplatin in PDX models for pancreatic acinar cell carcinoma. Lorbinectedin was tested to induce depletion in tumor-associated macrophages, which was an essential component of its in vitro synergism with gemcitabine, in pancreatic adenocarcinoma mouse models. Cai et al. also used orthotopic pancreatic PDX mouse models to test the anti-tumor effect of Apar S10.

Regarding the biomarker discovery for pancreatic cancer, the association between PDX models and human trials could provide sufficient information regarding drug susceptibility and drug resistance. Data have shown that the expression of the gemcitabine activating enzyme deoxycytidine kinase was a predictor of drug efficacy. A subsequent analysis of this marker in clinical samples confirmed these results. Moreover, in the same study, they found that the pre-treatment levels of deoxycytidine kinase protein were most associated with the overall survival rate and were stable among the matched pre-treatment and post-treatment tissues.

The efficacy of trastuzumab in pancreatic cancer with high human epidermal growth factor receptor 2 (HER-2) expression was studied in PDX models. Clinical application of trastuzumab is expected in pancreatic cancer with 3+ HER-2 expression.

A study assessed the efficacies of the inhibitors for epidermal growth factor receptor/HER2 receptors and the downstream KRAS effectors, mitogen-activated protein kinase/extra-cellular signal-regulated kinase kinase 1 and 2, on pancreatic cancer tumor cell proliferation in a murine orthotopic xenograft model. The result provided a rationale for achieving the co-inhibition of these pathways in the treatment of pancreatic cancer patients. CTC could be evaluated as a biomarker in the study of the phosphatidylinositol-3-kinase inhibitor, BKM120. PDX mouse models in pancreatic cancer indicated mutational analysis of CTCs, and serial monitoring of CTC burden may be used as a minimally invasive approach to predict and monitor treatment response to guide therapeutic regimens.

The discovery of resistance biomarkers is equally important. Compared to the gemcitabine-sensitive cells, the gemcitabine-resistant cells had a higher level of MCF2L expression, suggesting that MCF2L plays an important role in gemcitabine resistance in the PDX models. Das Thakur et al. generated a vemurafenib-resistant melanoma PDX model. They found that the mutation in BRAF is a critical factor for continuous vemurafenib resistance.

Based on these data, PDX models may play an important role in drug-response studies to help in selecting patients who are most likely to be sensitive to a new agent and prioritize the development of new biomarkers.

Bio-information of pancreatic cancer

PDX models could be used to study the bio-information (molecular mechanism, signaling pathway, and mutations)
of pancreatic cancer, such as the generation, suppression, and metastasis of cancer and the impact of the tumor microenvironment. CXCL12 reintroduction significantly reduced tumor growth in vitro, with significantly smaller tumors in vivo, leading to a pronounced survival advantage in a pre-clinical model. These data discovered that the normal expression of CXCL12 plays a functional tumor-suppressive role in pancreatic ducts and had the functional tumor suppressive ability to regulate both tumor growth and cellular dissemination to metastatic sites.[54] Investigating tumors within a relevant microenvironment provide the information for cancer cell-stromal interactions and uncovers potential therapeutic targets within the microenvironment. For example, Olive et al.[55] demonstrated that IPI-926 could target the pancreatic cancer microenvironment by inhibiting the Hedgehog cellular signaling pathway to improve drug delivery, efficacy, and survival. Walters et al.[25] observed that the mice bearing pancreatic xenografts frequently developed liver, diaphragmatic, and peritoneal metastases, with local retroperitoneal invasion. Pergolini et al.[32] identified that the clinical and pathological factors associated with successful tumor engraftment and xenograft growth rate and the successful establishment of PDAC PDX predict an increased risk of disease recurrence and mortality in the original patients. This xenograft model allowed the comprehensive investigation of genetic and molecular pathways to drive metastatic disease and directly test new therapeutic strategies targeting metastasis. Prioritizing genomic alterations based on tumor-specific vulnerabilities is a conceivable approach to detect mutations in cancer cells. Identifying the association between these molecular vulnerabilities will produce a comprehensive catalog of the potential therapeutic targets for cancer and provide a rationale for patient classification. The PDX models were considered beneficial in detecting molecular vulnerabilities and mutations.[56]

**Personalized medicine**

As we enter the era of “precision medicine,” PDX models could meet the requirements for personalized medicine over the other pre-clinical models options.[57] In clinical settings, the concept of precision medicine is defined as grouping patients into sub-group based on personal tumor biology and sophisticated genomic profiling to enable certain therapies targeting the sub-group or even the individuals.[58] Contrary to the conventional chemotherapy, the precision medicine combines individual patient’s characteristics, with most appropriate chemotherapy, molecularly targeted agents, or other therapeutics to maximize treatment efficacy and minimize side effects.[59-61] To meet these requirements, PDX model is an appropriate pre-clinical model as it preserves the tumor biology of individual tumor and represents the characteristics of a sub-group with similar genetic profile. Moreover, PDX model can even recapitulate heterogeneity within the same tumor specimen (intra-tumoral heterogeneity). A report showed the effectiveness and selectivity of the identified treatment responses for more than 500 regimens for single and combination drug regimens and suggested that sensitivity profiling of PDX models could inform
personalized therapy design for pancreatic cancer. A study showed that the gene expression profiling of patient-derived pancreatic cancer xenografts predicts sensitivity to the bromodomain and extra-terminal family of proteins inhibitor JQ1 and the value of PDX models in pancreatic cancer personalized treatment.

**Limitations and Challenges in Pancreatic Cancer PDX Models**

Although PDX model is a relatively ideal pre-clinical model for pancreatic cancer therapy development, it has also some limitations. First, the source of specimens from surgery resection is the most common, which guarantees the success rate of implantation. A great effort should be made to improve the success rate of specimen from biopsy, ascetic fluid, and other sources. More research on the technology using small specimen is required as well. Second, the PDX model should be careful on the selection of the tissue section for implantation, and only the most appropriate tissue comprising the essential elements for PDX models should be implanted. The pancreatic tumor, as a type of solid tumor, specifically some large tumors, needs to be elaborately sorted before the transfer from patients’ tissue. In some cases, stromal cells, such as fibroblasts, and vessel cells are implanted by mistake; undoubtedly, the results generated from these models were unreliable, which is a significant burden for the patients waiting for the results of the test therapies. Third, the PDX model has slow growth and low take rate, considered as the biggest limitation of PDX model. It usually takes 2 to 8 months to develop the model, but it is too long for pancreatic cancer patients with low survival expectation. Some patients even would die before the final development of PDXs. Fourth, the host mouse is immune-deficient to avoid the rejection of human cancer tissues. Hence, the conventional PDX model is not appropriate for evaluating the response of immune-modulating agents, and PDX models with entire or part of the human immune system are required to assess immune-oncology therapies in pre-clinical research. Fifth, although PDX models can well preserve the characteristics of pancreatic cancer as mentioned above, there is no doubt that the PDX tumor will change with the passage. Recent studies have shown that clonal selection occurs in propagation steps; hence, PDX models nearly but do not equally predict clonal selection. Finally, as mentioned above, human stromal components are replaced by murine elements in PDX model, specifically for the subcutaneous implantation. Hence, PDX model cannot totally mimic the microenvironment of human pancreatic cancer.

**Perspective of Pancreatic Cancer PDX Models**

Next-generation pancreatic cancer PDX models would use the genetically modified humanized mice. Conventional PDX models are developed in immunocompromised mice that fail to screen for immune-modulating agents. To investigate immunity-cancer interactions and pre-clinical assessment of cancer immune therapies, which require PDX models with human immune system, it is necessary to establish a human immune-conditioned PDX models. Next-generation PDX models would be more efficient for developing new therapeutic drugs. As a way to increase the PDX innovation, new models need to be developed. MiniPDX (Shanghai LIDE Biotech, Co., Ltd., China), one of the new models, is currently under investigation. However, further researches are still required to verify its validity. Next-generation PDX models are still needed to simulate real cancer-stromal interactions in patients. PDX may be a better choice, but it has technological limitations. The most important one is the lower success rate compared to subcutaneous implantation or renal capsule implantation. PDX models can be used in co-clinical trials. Because the PDX models preserve pancreatic cancer tumor biology, PDX models could be developed from patients enrolled in clinical trial and subsequently treated with the same regimen to monitor clinical response. Heid et al. have performed co-clinical assessment of tumor cellularity in pancreatic cancer in GEMMs. Hence, the use of PDX models in the co-clinical trial may be increased in the future because they reflect personalized medicine in a pre-clinical setting. As mentioned above, PDX models could be used to study the characteristics of pancreatic cancer. The PDX models essentially provide important in vivo and in vitro evidence to help in the basic research of cancer, from tumorigenesis, metastasis, to recurrence. This aspect will be explored further.

**Conclusions**

PDX models are widely used in pancreatic cancer research as it better preserves tumor features than the other models. It is an important tool for studying cancer biology, biomarker development, and drug screening and also a route for personalized medicine. In this review, we outline the status and application of PDX models in both basic and pre-clinical pancreatic cancer researches. We believe that the PDX model is one of the most appropriate pre-clinical tools that can improve the prognosis of patients with pancreatic cancer in the future.

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**Conflict of interest**

None.

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