REVIEW ARTICLE

Progress in Animal Models of Pancreatic Ductal Adeno-carcinoma

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ABSTRACT

As a common gastrointestinal tumor, the incidence of pancreatic cancer has been increasing in recent years. The disease shows multi-gene, multi-step complex evolution from occurrence to dissemination. Furthermore , pancreatic cancer has an insidious onset and an extremely poor prognosis, so it is difficult to obtain cinical specimens at different stages of the disease, and it is, therefore, difficult to observe tumorigenesis and tumor development in patients with pancreatic cancer. At present, no standard protocols stipulate clinical treatment of pancreatic cancer, and the benefit rate of new targeted therapies is low. For this reason, a well-established preclinical model of pancreatic cancer must be established to allow further exploration of the occurrence, development, invasion, and metastasis mechanism of pancreatic cancer, as well as to facilitate research into new therapeutic targets. A large number of animal models of pancreatic cancer are currently available, including a cancer cell line-based xenograft, a patient-derived xenograft, several mouse models (including transgenic mice), and organoid models. These models have their own characteristics, but they still cannot perfectly predict the clinical outcome of the new treatment. In this paper, we present the distinctive features of the currently popular pancreatic cancer models, and discuss their preparation methods, clinical relations, scientific purposes and limitations.

INTRODUCTION

According to NIH statistics, the 5-year survival rate of patients with pancreatic cancer between 2009 and 2015 was only 9.3% in US (https://seer.cancer.gov/statfacts/html/pancreas.html). As such, pancreatic cancer is associated with the worst prognosis of any malignancy because it has an insidious onset, high malignancy, special anatomical location, low resection rate, and high recurrence rate, as well as lack typical symptoms. Furthermore, the incidence of the disease increases annually: by 2030, patients with pancreatic cancer are expected to outnumber those with breast and colorectal cancer in United States, and pancreatic cancer is projected to become the second most common cancer worldwide [1].

Owing to the characteristics of pancreatic cancer, it is difficult for clincians to obtain samples at different stages and to continuously observe the occurrence and development of pancreatic cancer in individual patients. For this reason, animal models of pancreatic cancer help clinicians to further understand the occurrence, development, invasion, and metastasis mechanisms of this disease [1], and can even be used to explore new therapeutic means.

In 1941, Wilson discovered that a diet supplemented with 2-acetylaminofluorene induced pancreatic cancer in albino rats [2]. By the late 20th century, as the incidence of pancreatic cancer increased, the study of animal models began to develop, with the help from government agencies.

An ideal animal model of pancreatic cancer should have the following characteristics: (1) A biological development process similar to that of human pancreatic cancer, which is stable and repeatable. Specifically, Pancreatic Ductal Adenocarcinoma (PDAC) mostly develops from precursor lesions, the most common type being ductal intraepithelial neoplasia (PanINs) [3]. Genetic mutations highly correlated with this process have been reported in the literatures [4]. At present, a series of mouse pancreatic cancer models have been constructed using genetic engineering technology. By mutating *KRAS, CDKN2A, TP53, SMAD4,* and other genes,

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researchers can induce ductal intraepithelial neoplasia, and the number of mutant genes is highly correlated to the severity of disease [5]; (2) Malignant phenotype similar to human tumors, such as anti-apoptotic effect, immune escape, and invasion and metastasis. A wide variety of pancreatic cancer cell lines are available on the market, with the phenotype and genotype of each representing a specific subtype of pancreatic cancer. Researchers can infer the mechanism of tumorigenesis and development by studying the relationship between the expression of different specific proteins in cell lines and tumor growth, invasion and metastasis; (3) An experimental method that is easy to implement and efficient in terms of labor and time, as well as a short model establishment period. In particular, pancreatic cancer models used in clinical studies of individualized treatment must have a high success rate and be suitable for large-scale preparation to ensure that they provide evidence regarding individualized treatment options for patients with a short survival time.

Spontaneous Tumor Animal Models

As used herein, the term "spontaneous tumor" refers to a specific tumor induced spontaneously in a laboratory animal using a chemical, viral induction, or experimental genetic techniques. This contrasts with a transplanted tomor. Spontaneous tumors are more similar to human tumors, so results from animal models of such tumors can be more easily extrapolated to humans. However, the occurrence of spontaneous tumors may vary, so it is difficult to obtain a large amount of tumor material in a short period of time. Moreover, the observation time is long, and the experiment is expensive.

Chemically Induced Animal Models

Rat: Wistar and Lewis rats are injected intraperitoneally with azaserine to induce acinar cell carcinoma of the pancreas, with liver, lung and lymph node metastasis [6, 7]. However, the lesions in this model lack a typical duct-like structure and often occur alongside tumors of other organs (mammary, liver, kidney). The chemicals 4-hydroxyaminoquinoline-1-oxide [8], nafenopin [9], clofibrate [10], N -(N-methyl-N-nitrosamide)-L-ornithine [11], and different N-nitro compounds [7] can induce acinar cell lesions without a duct-like structure. Vesselinovitch *et al*. found that topical benzopyrene can induce adenocarcinoma in rats. They implanted dimethylbenzanthracene crystal powder into the pancreas of Sprague-Dawley rats, and approximately 80% of them developed spindle cell sarcoma and poorly differentiated adenocarcinoma. Other researchers using this method have found ductal cell proliferation, tubular adenocarcinoma, acinic cell carcinoma, fibrosarcoma, and invasive ductal adenocarcinoma.

Hamster: Hamsters are one of the best animal models for inducing pancreatic cancer. For instance, some carcinogens that work in hamsters are ineffective in other animals, such as rats, mice, Dutch pigs, and rabbits. N-Nitroso-bis(2-oxopropyl)amine (BOP) has the highest specificity in this regard [12, 13], and it show a specific affinity for the pancreas, although its mechanism has not yet been confirmed. This N-Nitroso-BOP model shows unique characteristics that are similar to a well-characterized series of morphologic changes that occurs in the human pancreatic duct, and it frequently shows point mutations in codon 12 of the *K-ras* gene, concurring with findings in human pancreatic cancer [14, 15]. Meijers found that the early pseudoductular lesions, induced by BOP in the exocrine pancreas of hamsters originate from proliferating ductal/ductular acinar cells rather than proliferating dedifferentiated acinar cells [16]. In addition, the tumors induced in hamsters are most similar to human tumors in terms of morphology, clinical features, and biological manifestations. Not only benign and malignant tumors but also some rare lesions occurred in hamsters. Tumors in hamsters, just as in humans, may show perineural invasion, involvement of the lymph nodes adjacent to the pancreas, weight loss, diarrhea, ascites, and thrombosis. Occasionally, the tumors also involve jaundice,because they mainly occurr in the body and tail of the pancreas. Similar to human tumors, serum antigens CA125, 17-1A, TAG-72, TFGR-α, EGFR, and lectin have been detected in hamster pancreatic tumors, and glucose tolerance has been observed. However, carcinoembryonic antigen, pancreatic cancer embryonal antigen, and α-fetal protein are low or unexpressed [17]. Animal models like the hamster model of pancreatic cancer can help identify known and emerging human risk factors and implement appropriate interventions.

Genetically Engineered Mouse Model of Pancreatic Cancer

Many recent studies have used genetic technology to introduce oncogenes into mouse embryonic or somatic cells through tissue-specific promoters targeting the pancreas and inducing pancreatic cancer. Genetically Engineered Mouse Models (GEMMs) are constructed using transgenic, gene knock-in, and gene knock-out techniques to transfer specific genes into mice *via* retroviruses. Most currently used GEMMs are developed using KRAS proto-oncogenes. The transgenic mice that overexpress the mutant KRAS gene can mimic pancreatic tumorigenesis [18]. As most human pancreatic cancers are ductal adenocarcinomas, researchers preferred the selected promoter to be limited to the ductal epithelial or exocrine cells. Most single genetically modified models cannot reproduce the whole process of pancreatic tumorigenesis, and the progression from the normal epithelium to cancer cells often requires gour to five genetic mutations [19]. Additional genetic modifications, such as P53 and P16 inactivation, can accelerate tumorigenesis and metastasis. Conditional gene knockout technology allows gene modification to be limited to a certain part or a certain stage of development, so the time and space of the mutant gene can be accurately contolled, enabling more accurate study of gene function. The Cre/loxp recombinase [20] and tet on systems [21]

are the most commonly used conditional gene knockout strategies [22]. GEMMs of pancreatic cancer are similar in nature to the human disease. In particular, their metastasis pattern is the most similar to that of human pancreatic cancer. The model can be used to study early-stage tumor formation, allowing researchers to ascertain tumor pathogenesis and the effects of therapy. However, the model is limited because it is genetically and biologically different from the human tumor, its modeling time is difficult to control, and its cost is high. Furthermore, it is difficult to meet experimental requirements in terms of quantity.

Tetracycline-Induced TetO-Cre

Cre expression can be activated when rtTA or tTA with transcriptional activation functions bind to tetO. Binding of rtTA or tTA to tetO is regulated by tetracycline or its derivative doxycycline (Dox). Specifically, tTA only induces Cre expression when it binds to tetO in the absence of Dox; it does no bind to tetO when Dox is present, so Cre is not expressed in such cases. Convasely, rtTA binds to tetO and induces Cre expression when Dox is present; when Dox is absent, it does not bind to tetO, and Cre is not expressed **(Figure 1)**. Thus, in tetO-Cre and tissue-specific rtTA (or tTA) double-transgenic mice, Cre recombinase can be controlled in space and time by administering or withdrawing Dox. Cre recombinase specifically recognizes the loxp site and cleaves the DNA sequence, causing DNA sequence recombination between the two sites.

Establishment of Animal Models Based on Cell Lines

To understand certain aspects of human pancreatic tumors, such as tumor growth, metastasis, drug efficacy, etc., researchers generally prefer nude mice with T-cell defects. The phenotype of the original tumor can be maintained after cancer cells of human origin have been implanted into such models, although some abnormal reactions will occur [23]. However, one recent study used some combined immunodeficiency mice as hosts to receive pancreatic cancer cells of human origin. The results showed that differences in immunodeficiency do not affect the occurence of pancreatic cancer in mice, and that the potential for metastasis is largely determined by the specific cell line [24].

Cell Line Selection

The low diagnostic rate of pancreatic cancer is partly due to a lack of specific molecular changes, so it may be useful for researchers to understand their known cell lines **(Table 1)**. Therefore, before beginning studies on pancreatic tumors, researchers should know what the research direction is. This will allow them to select the appropriate cell line and evaluate its clinical background, growth characteristics in both *in vitro* and *in vivo* experiments, and the phenotypic characteristics (adhesion, invasion, metastatic ability [25]), and genotypic changes, which most often occur in the *KRAS, SMD4, TP53*, and *P16* genes [26, 27, 28, 29] **(Table 2)**.

Cell Geonotypes: Studies have shown that mutations in these four genes are not associated with the degree of differentiation [30] or biological behavior [31] of pancreatic cancer cells. However, research does indicate that *in vivo* tumor metastasis is related to alterations in *hte P53* gene, suggesting that genotype is related to the phenotype in pancreatic cancer cell lines [32, 33].

Cell Metastasis and Invasion: The biological characteristics of tumor metastasis can be understood through cancer cell metastasis experiments. In the Boyden chamber invasion model, cells migrated from one chamber to another through the artificial basement membrane pores at different chemokine concentrations [34]. Other migration experiments include the transwell and scratch assays [35]. Stahle *et al*. found that PANC-1 cells were five times more active than BxPC-3 in the transwell migration experiment [36]. Lin *et al*. evaluated mobility by measuring the phagocytic trajectory of cell movement on a colloid surface; they found that both HPAF-II and BxPC-3 cells had good mobility [37].

Tumorigenicity: In a study by Schmidt, a pancreatic cancer cell suspension was injected into nude mice. The researchers then observed the volume, quantity, and metastasis of the subsequent tumor to roughly ascertain the tumorgenicity of the cell line. Relatedly, different methods of tumor induction can cause differences in the tumor formation rate and metastatic colonization location. For example, intra-abdominal or intravenous injection, *in situ* implantation, and implantation metastasisshow differing outcomes. Subcutaneous injection of tumor

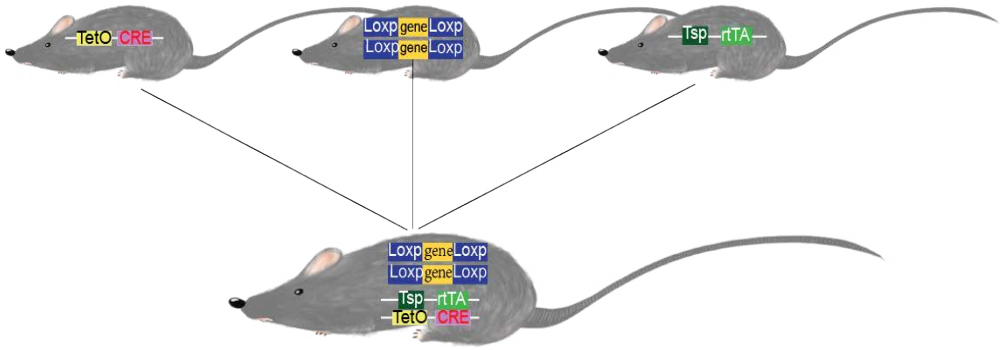

Figure 1. Tetracycline-induced TetO-Cre for GEMM. Cre mice (TRE-Cre, also called tetO-Cre) controlled by a tetracycline-responsive element (TRE, also called tetO). Mice expressing a tetracycline-responsive transcriptional activator rtTA or tTA driven by a tissue-specific promoter.

Table 1: Pancreatic cancer cell lines.

Human pancreatic cancer cell line							
Cell line	**Tissue origin**	**Metastasis**	**Doubling time**	**Differentiation degree**	**Morphology**	**Tumor formation rate (subcutaneous)**	**Ref**
AsPC-1	Ascites	Yes	38-40 hrs	Poor	Epithelioid		[69]
HPAF-II	Ascites	Yes	42 hrs	Moderate	Epithelioid		[70]
HPAC-1	Primary tumor	-	41 hrs	Good	Epithelioid		[71]
MIA PaCa-2	Primary tumor	-	40 hrs	Poor	Epithelioid	66%	[72]
PANC-1	Primary tumor	Yes	52 hrs	Poor	Epithelioid	86%	[73]
BxPC-3	Primary tumor	No	48-60 hrs	Moderate-Poor	Epithelioid	100%	[74]
Capan-2	Primary tumor	No	96 hrs	Good			[75]
Capan-1	Liver Metastasis	Yes	-	Good	Epithelioid		[76, 77]
SU.86.86	Liver Metastasis	Yes	77 hrs	Moderate-Poor	Epithelioid		[78]
CFPAC-1	Liver Metastasis	Yes	31 hrs	Good		100%	[79]
Suit-2	Liver Metastasis	Yes	29-38 hrs				[80, 81]
SW1990	Splenic Metastasis	Yes	64 hrs			100%	[82]
Hs766T	Lymphatic Metastasis	Yes	6-7days	-	Epithelioid		[83]
Colo357	Lymphatic Metastasis	Yes	21 hrs	Good			[84]
T3M4	Lymphatic Metastasis	Yes		Moderate			[85]

Animal-origin (hamster) pancreatic cancer cell line				
Cell line	**Carcinogen**	**Differentiation degree**	**Gene mutation**	**Ref**
PC1	BOP	Good	*K-ras, P53*	[86, 87]
WDPaCa	BOP	Good	*P53*	[88]
PDPaCa	BOP	Poor	*k-ras*	[88]
HPC	BOP	Poor		[89]
HP1	BOP			[90]
HaP-T1	BOP	Good-Moderate		[91]
H2T	BHP		*K-ras, P53*	[92, 93]
HPD(1-3)NR	BHP	Moderate	*K-ras, P53*	[91, 94]
Pan02	MCA		*K-ras,smad4*	[95]

Note: BHP: N-nitrosobis(2-hydroxypropyl)amine; animal-origin pancreatic cancer cell lines are commonly used in inbred mice of the same origin for allogeneic transplantation. This model is used more frequently in tumor immunology studies and to evaluate single-agent or combination immunotherapy studies.

Table 2. Expression of mutant genes in cell lines.

Gene	Expression of Cell Line
KRAS	Occurred in almost all of the primary tumors of pancreatic cancer, but the BxPC-3 cell line is WT
SMD4/DPC4	Capan-2, MIA PaCa-2, PANC-1, SU.86.86 without SMD4 gene inactivation
TP53	Its mutation occurs in 50% of pancreatic malignant tumors and is associated with late tumor progression
CDKN2A/P16	Basically all pancreatic cancer cell lines have inactivation of the *P16* gene

cells is the most common experimental method, probably because it is easy to operate. Different cell lines result in tomors of significantly different sizes. In one study, Capan-1, PANC-1, and MIA PaCa-2 cell suspensions were injected into the Severe Combined Immunodeficiency (SCID) mice. After 30 days, a biopsy was taken, revealing the tumor sizes in the following oder: MIA PaCa-2 > Capan -1 >PANC-1 [38]. Eibl *et al*. [39] uesd donor nude mice to grow Capan-2 and MIA PaCa-2 tumors. They then removed the tumor, cut it into a cube of 1×1×1 mm^3, and implanted it in the pancreatic tail of recipient nude mice. They reported a 100% tumor formation rate and that MIA PaCa -2 tumors grew faster. However, because the tumor was first formed under the skin, this *in situ* tumor implantation model lacks the changes related to the tumor microenvironment and morphology of early-stage tumor. Direct injection of cancer cells into the pancreas can better reflect the tumorigenesis and development of pancreatic cancer. Indeed, several studies have focused on direct injection of different pancreatic cancer cell lines into the pancreas of SCID mice to induce tumor formation [25]. The tumor gomation rate were as follows: AsPC-1, 100% (10/10); CFPAC-1, 100% (10/10); HPAF- II, 100% (8/8); Capan-2, 90% (9/10); Hs 766T, 90% (9/10); HPAC, 88% (7/8); PANC-1, 80% (8/10); and BxPC-3, 67% (6/9).

Establishment of a Transplanted Tumor Model

Subcutaneous Tumor Formation: This model involves planting tumor cells or tumor tissue directly under the skin of mice. Nude or other immunodeficient mice are generally used in such experiments to study the biological behavior of tumors and intervention therapy. The model is easy to operate, inflicts little trauma on the mice, and confers a high tumor formation rate (80%-100%). The

implantation sites are usually located in the back, neck, armpits, groin, or other areas with a rich supply og blood and lymphatic vessels. The model uses tumor cells in the logarithmic growth phase. Briefly, the cell suspension density is adjusted to 1-2×10^7/mL using PBS, and the cell suspention is injected into the implantation site at a volume of 0.2 mL. The mice are then fed in cages. The tumor formation rate and size differ depending on the cell line used. Although subcutaneous tumor formation is easy to operate and suitable for large-scale experiments, it is limited to subcutaneous growth, without distant metastasis, or internal organ invasion, and it cannot truly reflect the tumor microenvironment of pancreatic cancer. In this way, the model does not match the real human pancreatic cancer, and it is therefore used to assess the response of tumors to specific drugs, including antibody-based and cellular drugs, but not for mechanism studies.

In situ Tumor Formation: *In situ* pancreatic cancer can be induced using *in situ* injection or pancreatic capsule implantation of tumor cells. In the latter case, tumor cells grow subcutaneously for 4 weeks to form a tumor. The tumors are then excised and cut into pieces of 1~2 mm^3. In recipient mice, the pancreatic capsule is then opened, and the tumor is implanted into the tail of the pancreas. The tumor formation period is 4 weeks, and the rate is 100%; the injection of tumor cell suspension has a lower tumor formation rate than the transplantation method, and the injection port is likely to cause cell shedding, resulting in extensive transplantation metastasis. For this reason, the method is rarely used [40]. However, researchers have implanted pancreatic cancer cells into a recently developed thermosensitive biogel. The cells then develop into tumors. The gel is liquid at a low temperature and turns into jelly at body temperature, which prevents cell shedding; the gel can also dissolve any intervention drugs and is an excellent model for studying such drug. In general, *in situ* tumor formation of pancreatic cancer can fully simulate the internal environment of tumorigenesis and development, and it can affect the whole body during the tumor evaluation period. With the *in situ* tumor model, the tumorigenesis time is short and the tumorigenesis rate is high, so the original tumor structure is maintained, as are most biological characteristics of the human tumor, including the growth of primary tumor, local invasion, and subsequent distant visceral metastasis. The model is an indispensable for studying the tumor microenvironment and is important for exploring new surgical approaches, nutritional support, and other ancillary treatments for pancreatic cancer.

Liver Metastasis Model: At the time of presentation, patients with pancreatic cancer are usually at an advanced stage, with tumor invasion into adjacent structures or metastasis into the peritoneum *via* direct extension, as well as into the regional lymph nodes or distant organs, such as the liver and lungs [41]. The most commonly used liver metastasis models involve spleen injection and direct intrahepatic implantation. In such models, the spleen is injected with a pancreatic cancer cell line at the logarithmic phase, and a 1×10^6/mL single-cell suspension is prepared using ice-cold sterile PBS. Experimental animals are then anesthetized and disinfected, and the spleen is exposed at a distance of 0.5 cm left of the ventral midline. Next, 100 μL of cell suspension is injected slowly using an insulin syringe. Immediately after injection, tissue glue or an alcohol cotton ball are used to prevent bleeding and transplantation metastasis into the abdominal cavity. This liver metastasis model is mainly used to study the invasive ability of pancreatic cancer; it is not applicable to the study of blood flow dissemination. The intrahepatic implantation model is a supplement to the model. In this model, the tumor cell suspension is directly injected into the liver through the portal vein. Tumor tissue from human or experimental animals can then be cut into a 1-mm^3 tumor mass and directly implanted under capsule of the left lobe using a 16-gauge needle. The above models can complement each other and be used to systematically study various cascade processes in which pancreatic cancer develops from the primary tumor, invades and migrates into the blood vessels, and acclimates the microenvironment of the metastatic tumor, allowing the secondary tumor to grow.

Lung Metastasis Model: The lung metastasis model is established by injection of tumor cells through the tail vein. After the tumor cells enter the capillary network of the lungs through the systemic circulation, they gather in the microvessels of the lungs, and metastatic tumors 1~2 mm in diameter are formed in the lungs after around 1 month. By labeling tumor cells with fluorescent proteins, tumor colonization and growth can be continuously observed under an *in vivo* imaging system. This method also causes tumor formation in ograns other than the lungs, such as the liver, so this method is also used to study the hematogenous metastasis.

Lymph Node Metastasis Model: The presence or absence of lymphatic metastasis has a guiding role in the treatment of pancreatic cancer, but no imaging method or technique can satisfactorily track lymph node metastasis [42, 43]. Therefore, to better study this phenomenon, a stable lymph node metastasis model for pancreatic cancer is needed. No cell lines have been reported to confer specific lymph node metastasis, and researchers usually screen for such cell lines by continuous screening and planting *in vivo*. For example, Li *et al*. used the BxPC-3 cell line to produce a highly lymphatic metastatic pancreatic cancer cell line, dubbed BxPC-3-LN5, through repeated screening. They then injected 100 μL of 1×10^9/mL cell suspension into the left hindpaw of BALB/C nude mice and observed swollen lymph nodes in the popliteal fossa of the left knee after about 5 weeks [44].

Perineuronal Invasion Model: Patients with pancreatic cancer often have severe pain due to peripheral nerve invasion, which considerable impacts quality of life. Pancreatic cancer has a high incidence of invasion and metastasis into the nerves and plexuses surrounding

the arteries, and this is one e important factors in local recurrence of pancreatic cancer after excision. Therefore, reseachers must further explore perineuronal invasion of pancreatic cancer, with a view to reduce patient suffering and improve clinical treatment. Both human and mouse perineuronal invasion models of pancreatic cancer are used. In the former case, the celiac plexus and superior mesenteric artery nerve are obtained from a donor 6 hours after death by postmortem autopsy. Under aseptic conditions, the nerves are then cut into 1 cm pieces and immediately placed in RPMI-1640 medium containing antibiotics. The isolated tissues are implanted subcutaneously in non-obese diabetic (NOD)/SCID mice. After 4 weeks, 7×10^6 pancreatic cancer cells are injected near the plantation site. After 5 to 8 weeks, the tumor volume is around 1.5 cm^3. The mouse model also uses NOD/SCID mice: 7×10^6 pancreatic cancer cells are injected into the midline of the mouse. In this model, it is better to choose a cell line with a tendency towards perineuronal invasion, such as Capan-1 or Capan-2 [45, 46].

Patient Derived Tumor Xenografts (PDTX)

In this model, researchers implant small tumors from a patient's pancreas into experimental immune-compromised mice, simulating their native growth environment [47, 48]. Tumors cultured using this method can better preserve matrix heterogeneity and retain more human tumor matrix components in the early generations (within 10 generations) [49]. They can also retain the histological characteristics of the original tumor, such as morphology, lymphatic and vascular systems and necrotic areas [50]. Moreover, they retain molecular diversity, with at least the first 10 generations showing microarray-comparative genomic hybridization, microsatellite instability, and higher genetic stability—genesequencing shows that neither the DNA copy number nor the gene expression profile differs significantly between the early and late generation models [51]. This model can reflect the tumor characteristics in individual patients and is necessary to study individualized treatment. However, the cycle time is long and the model's success rate is low. In addition, the most typical feature of pancreatic cancer is rich stromal cells. With the passage of the tumor, the human stromal cells in the tumor are gradually replaced by the mouse cells, so they still cannot truly reflect the original biological behavior.

Establishment and Application of Pancreatic Cancer Organoid

Cell lines, genetically engineered mouse models and transplanted tumor models all have important clinical significance and scientific research value, but each also has clear shortcomings, especially with regards to individualized treatment. The establishment of xenograft tumors requires effort and time, as well as materials. In addition, *in situ* tumor models based on cell lines never truly reflect the patient's condition. Organoid models are artificially controllable and can reproduce the three-dimensional structure of PDAC; it has attracted increasing attention because it can overcome the limitations of the traditional model. Organoids can be used to study tumorigenesis and tumor development, including the solid and interstitial components of the tumor, and also as a "test bed" to help determine specific treatment options for patients using *in vitro* testing.

In vitro culture of the pancreas can be traced back to 1938, when Carrel and Lindberg used the irrigation method to culture a cat's pancreas *in vitro* for 4 weeks [52]. In the 1980s, researchers began to explore how to culture isolated pancreatic cells in a three-dimensional structure [53]. On the basis of previous experience, Speier *et al*. sliced the pancreas of the mouse and then successfully cultured it for 7 days in agarose [54]; the normal human pancreas and pancreatic tumors can be cultured in the same way for 6 days [55]. In a further improvement of this method,part of the normal pancreas and tumor were placed in a collagen or matrix gel and used for drug sensitivity testing [56]. In addition, PDAC cell lines have been directly cultured in a three-dimensional structure [57], using various physical methods to prevent cell adhesion and form a polarized spheroid structure. Lorenzo Moroni's team were aimed to investigate the interactions between human primary PDAC cells and take polymeric scaffolds with different design and composition to create biomimetic models of PDAC [58]. The cultivation of pancreatic cells in a three-dimensional space has allowed researchers to realize the possibility of organoids, but no uniform definition of organoids has yet been agreed.

Clevers *et al*., working with Tuveson Laboratories [59], found that cells isolated from PDA or PanIN lesions in mice can be cultured into organoids. They prepared pancreatic ductal organoids from multiple murine primary tumors (mT) and metastases (mM). Orthotopic transplantation of mT organoids initially generated low- and high-grade lesions that resembled mPanINs . Over longer periods of time (1–6 months), transplants developed into invasive primary and metastatic mPDA. Similarly, this kind of tumor model is applicable to human pancreatic cancer cells. They researchers modified the culture conditions to support human normal and malignant pancreatic tissues. These Patient-Derived Organoids (PDO) can be cryopreserved and passaged indefinitely, and they can be genetically, transcribed, proteinized, and biochemically analyzed. Therefore, this system is an ideal model for exploring tumor progression at each stage . Melissa Skala *et al*. [60] used a similar method to isolate PDA cells in transgenetic mice with the following genotype: Ptf1a Cre/+; Kras LSL-G12D/+, Tgfbr2 fl/fl mice. These cells were cultured in mixed medium and serum-containing medium to develop into an organoid. This method can be used to culture tumors that have been removed from human pancreatic cancer.

Senthil Muthuswamy *et al*. [61] established three-dimensional culture conditions to induce differentiation of human pluripotent stem cells into exocrine progenitor cells, forming ductal and acinar structures *in vitro* and *in*

vivo; they also identified culture conditions for cloning freshly collected PDAC cells into tumor organoids, which can maintain the differentiation status, histological structure, and phenotypic heterogeneity of the primary tumor, as well as preserve the unique physiological changes seen in the patient, including hypoxia, oxygen consumption, epigenetic marks, and sensitivity difference to histone methyltransferase EZH2 inhibition.

Calvin Kuo *et al*. [62] used an "Air-Liquid Interface" (ALI) method in which embryonic tissue fragments were cultured in type I collagen gels built on a permeable substrate with a medium underneath that allows nutrients to diffuse from the bottom. The top of the medium was exposed to the air so that the cells could obtain a higher level of oxygen than in conventional culture methods, thereby preventing hypoxia. In the ALI culture, a pancreatic tissue from newborn mice formed an organoid surrounded by stromal cells and containing ductal epithelial cells. It could survive for 50 days without exogenous growth factors, but cannot be passaged. Later, the researchers cultured pancreatic organoids from $Kras^{LSL\text{-}G12D/+}$ and $Trp53^{fl/fl}$ mice.

In most organoid studies in the cancer field, primary carcinoma samples have been generated under Adult Stem Cell (ASC)-organoid conditions. However, CRISPR mutagenesis technology has been applied to Pluripotent Stem Cell (PSC)-based organoids to generate cancer-causing mutations. Organoid cultures allow several parameters to be observated: (1) Interpatient variation can be captured and maintained, (2) Patient material can be xenotransplanted with high efficiency, (3) The drug response of the corresponding patient can be faithfully reproduced, and (4) Drug sensitivities of PDOs can be recapitulated in PDX settings. The organoid model is highly efficient, so a corresponding organoid biobanks can be established on the basis on different tumor types. Indeed, several studies have reported that organoids can be derived from needle biopsies taken from liver cancer [63], pancreatic cancer [64, 65], or human colorectal cancer metastases [66]. In the studies of colorectal cancer, two laboratories separately have established human intestinal cell organoids containing mutant tumor suppressor genes and oncogenes, which can be used to study the mechanism of tumorigenesis and invasion [67, 68]. In the near future, pancreatic organoids will likely play a key role in the development of precision medical treatment against PDAC, which will have its own unique advantages [69-95].

CONCLUSION

Because pancreatic cancer shows no specific early clinical manifestations and has high mortality, medical researchers find it difficult to study the biological behavior and internal mechanisms of early pancreatic cancer, and our understanding of the mechanism underlying tumorigenesis is limited. Early diagnosis allows patients to receive timely treatment in the curable phase. Use of experimental animal models is an important method for gaining insight into the etiology, risk factors, prevention, and treatment of this tumor. This approach requires a model that is similar in biology, morphology, and clinical characteristics to human tumors. Although many mouse models can be obtained using transgenic technology, there is still a lack of specificity for clinical research.

Perhaps importantly, 70% of pancreatic cancers are induced by carcinogens, with nitrosamine and polycyclic aromatic hydrocarbons in tobacco being high risk factors for inducing pancreatic cancer. Therefore, to induce tumorigenesis of pancreatic cancer, chemically induced models are more useful. However, the transplantation tumor model has been used to study etiology, diet, modification factors, and some natural products, as well as early diagnosis, prevention and treatment of pancreatic cancer.

In summary, current animal models can mimic the characteristics of most human pancreatic cancers, but no model has become a “gold standard” that meets the needs of all research. By simply focusing on specific needs and combining the characteristics of each model, researchers can better study the overall process of tumorigenesis and development of pancreatic cancer. Ultimately, to reduce PDAC mortality, judgments based on genetic and non-genetic risk factors must be improved. As such, researchers must explore new biomarkers and high-resolution imaging techniques to screen for patients with early-stage, high risk cancer, and must carry out drug interventions to prevent PDAC progression and prolonging survival time. In the past few decades, improvements in animal models have driven advances in these areas, and these models will continue to make significant contributions in the coming years.

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Conflict of Interest

The authors disclose no financial relationships or conflict of interest relevant to this publication.

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