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Establishment and Advancement of Pancreatic Organoids

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Following the reporting of the first organoids, the development and application of tissue-specific organoids has expanded considerably in fields such as stem biology, regenerative medicine, oncology, biotechnology, and precision medicine. In addition, pancreatic organoids generated sequentially from pancreatic duct tissue, tumor tissue, and pluripotent stem cells, are increasingly used in research on stem cells, pancreatic islets, pancreatic ducts, type 1 diabetes, and pancreatic ductal adenocarcinoma. This article introduces organoids in general and reviews recent studies on the use of pancreatic organoids in particular.

Keywords: Organoids, Pancreas, Carcinoma, Pancreatic Ductal, Precision medicine

Introduction

The pancreas is an endoderm-derived organ that is composed of exocrine glands, which convert nutrients into small absorbable molecules, as well as endocrine glands called pancreatic islets that maintain blood sugar levels within a normal range. The exocrine glands contain acinar and ductal cells that secrete digestive enzymes and bicarbonate, respectively. Pancreatic diseases affect the intrinsic functions of this organ and can be life-threatening when the ability to absorb nutrients, neutralize stomach acid, and maintain blood sugar levels is impeded.

Since the first tissue-derived intestinal organoids were established [1], the field of organoid research has progressed and diversified substantially. Cells or tissues are harvested from the human body, cultured in 3D, and established as functional tissues or organs in vitro. Organoids are 3D structures that are primarily generated from stem cells or tissue-specific progenitor cells and resemble the structural and functional characteristics of a specific organ. Stem cells can divide, differentiate, develop into specified cells, and continuously self-renew; therefore, they are well-suited for organoid generation and ideal for cell-based therapeutic applications [2]. Pluripotent stem cells (PSCs) consist of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) that can differentiate into any cell type in the body depending on the conditions [3]. Adult stem cells are tissue-specific, can be isolated from mature adult tissues, and possess the ability to self-renew and differentiate into specific cell types [4,5].

These tissue-like 3D structures are generated from an increasing number of sources, including differentiated PSCs, fetal and adult primary tissues, and primary and metastatic tumors. The resultant organoid has been defined as "a three-dimensional structure derived from (pluripotent) stem cells, progenitor, and/or differentiated cells that self-organize through cell-cell and cell-matrix interactions to recapitulate aspects of the native tissue architecture and function in vitro" [6]. Organ-specific tissues generated from stem cells have been used to

form tissue- and organ-specific organoids [7]. Organoids are defined as small, self-organizing, 3D tissue culture structures generated from stem cells that can proliferate in vitro through various tissue culture procedures [2,6]. This article reviewed the establishment and recent advancements of pancreatic organoids.

Organoids

Highly proliferative tissues, such as the intestine, have substantial pools of progenitor cells that allow for regeneration during homeostasis or upon injury [8]. The first long-term 3D culture of intestinal organoids was reported to be generated using a single $Lgr5^+$ stem cell located in the intestinal crypts. The organoids were grown on Matrigel supplemented with various growth factors until they differentiated into multiple functional intestinal cell types [1]. Subsequently, human iPSCs and ESCs were differentiated into functional 3D intestinal organoids in vitro [9]. When liver tissue is damaged, only specific cells proliferate to regenerate the damaged tissue. Based on this characteristic, damaged mouse livers were extracted and cultured into 3D organoids by in vitro expansion of liver $Lgr5^+$ stem cells [10].

Unlike the intestine and liver, the pancreas does not regenerate after injury; however, pancreatic cells proliferate and exhibit pancreatic plasticity by islet δ- and α-cells conversion to β-cells, and acinar-to-ductal metaplasia, thereby exerting regenerative capacity [11]. Due to these properties, $Lgr5^+$ pancreatic ductal stem cells were induced by injuring mice pancreases with partial duct ligation or using an in vitro RSPO1-based culture to activate Wnt signaling to generate the first pancreatic organoids [12]. Under similar conditions, pancreatic and tumor organoids were generated from human pancreases and pancreatic ductal adenocarcinomas (PDACs), respectively [13]. Subsequently, pancreatic organoids were established from pancreatic progenitors differentiated from human PSCs (hPSCs) and tumor organoids were established from PDACs, the latter of which induce tumor-like changes by expressing gene mutations, exhibit tumor-like characteristics, and can be used as PDAC models for precision therapy [14].

Subsequent research has resulted in the consecutive development of the following organ-specific organoids [2,15]: gastric [16,17], retinal [18,19], cerebral [20], kidney [21-24], prostate [25], lung [26-29], cholangiocyte [30], trophoblast [31,32], skin [33], and blood vessel [34]. These organoid models are important tools for studying the development, regeneration, and replacement of normal tissues and organs as well as disease models for diagnosing, monitoring progress, and treating diseases such as cancer (Fig. 1).

Pancreatic research tools and pancreatic disease models

Various models are used in medical fields to better understand diseases. Although disease models such as 2D cell cultures, genetically engineered mouse models that allow for the introduction of targeted gene mutations, and xenografts have made substantial contributions to disease research, challenges remain in translating the laboratory results of these models to a clinical environment [2,35,36]. For example, species differences exist among cell lines that are cultured in vitro, in mice, or in humans [37]. Genetically engineered mouse models have a high success rate; however, this method involves a high time and cost commitment and often must be repeated in human models due to differences in genetic diversity and metabolism. Similarly, patient-derived xenograft is time-consuming and expensive to establish cell lines [2,38,39].

Commonly, 2D monolayer cell culture is used for drug discovery and various disease studies as it is considered to mimic in vivo cell growth [40,41]. However, cells maintained in 2D culture can lose cell-specific properties such as shape, polarity, differentiation, and metabolism because of the lack of cell-substrate and cell-cell interactions [40,42]. Furthermore, 2D pancreatic cell lines are limited in number and often genotypically altered in culture; therefore, they are often insufficient for studies on wide range of genotypes expressed by patients with pancreatic diseases, including pancreatic cancer [35,39]. This insufficiency is a key reason for the discrepancies between preclinical and clinical findings. Accordingly, 3D cell culture techniques were developed in which cells interact with their 3D environment [41], thereby allowing for organ-like structure modeling based on the cell type.

Conventional 2D cell cultures involve low cell-to-cell interactions, no soluble gradient, monolayered growth with a flat shape, and the attachment of one side of the cell to the surface of the container while the other side is exposed to the liquid [5]. In contrast, 3D cell culture incorporates increased cell-to-cell interactions, the presence of a soluble gradient, aggregate cells growth, and embedding of cells with three-dimensional attachments. Due to the increased cell-to-cell interaction and paracrine effects, stem cells are well differentiated and produce results similar to those found in vivo; however, the experimental methods involved are diverse and expensive [5].

Three-dimensional organoid models have primarily been

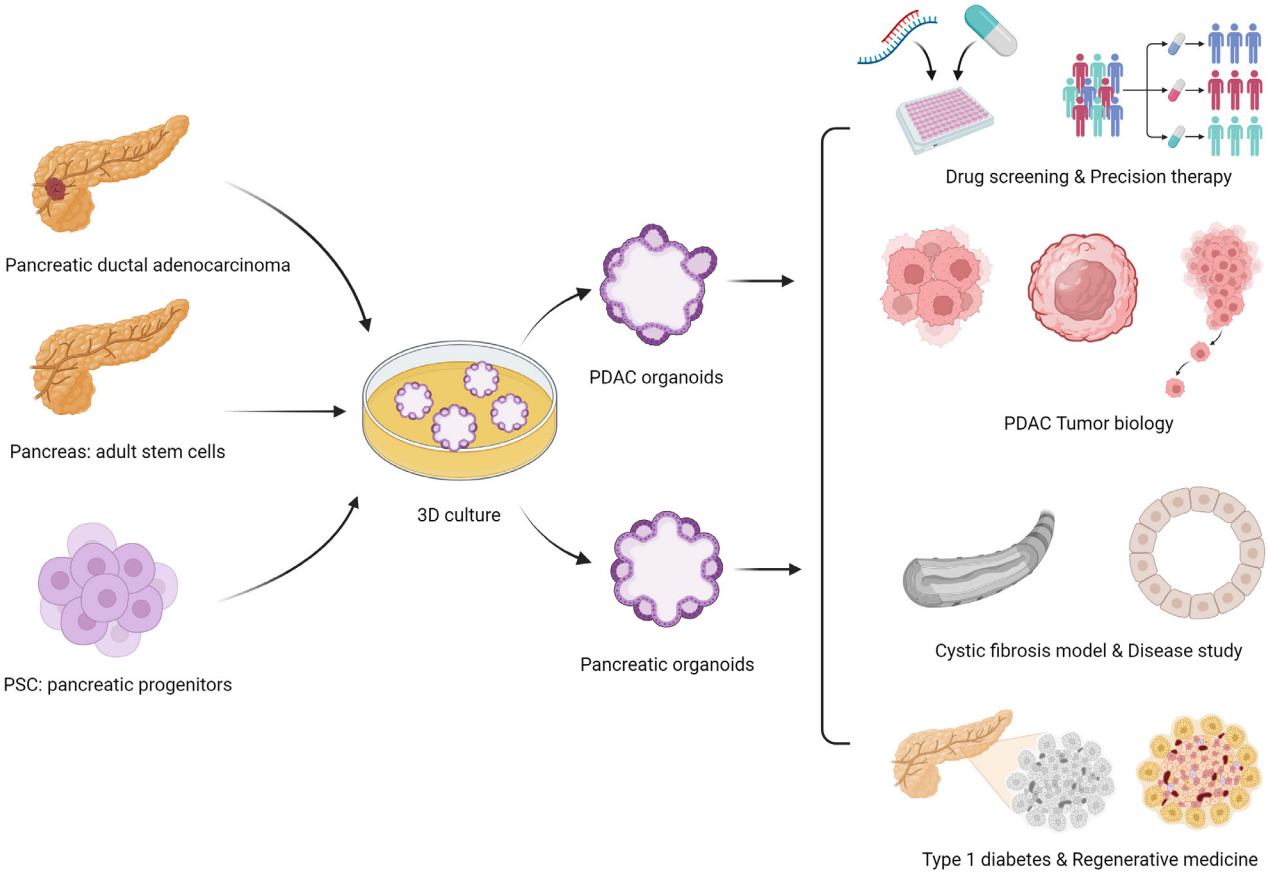

Fig. 1. Establishment and application of pancreatic organoids. Pancreatic organoids can be generated from adult stem cells of pancreatic tissue and pancreatic progenitor cells derived from PSCs, and pancreatic tumor organoids can be formed from PDAC. Pancreatic organoids can be cultured in 3D and subjected to various culture conditions, and manipulations such as genome editing can be performed during culture. The pancreatic organoids have applications in drug screening, precision therapy, PDAC tumor biology, type 1 diabetes, regenerative medicine, cystic fibrosis models, disease studies, and biobanks. PSCs, pluripotent stem cells; PDAC, pancreatic ductal adenocarcinoma. Figure created with BioRender.com.

used to study stem cells and diseases [43]. Along with the benefits of 3D culture, organoids mimic biological characteristics such as the 3D spatial arrangement of cells, cell-to-cell interactions, and various processes, thereby providing an organ system that is similar to the natural structure and properly preserves the genetic information critical for cancer research. The patient-derived organoid (PDO) and patient-derived xenograft models of patients with PDAC were comparable and had similar treatment responses [44]. In addition, PDOs could be used to predict patient response to treatment [45]. Compared to genetically engineered mouse models, PDOs are less time-consuming and costly, can be constructed from small amounts of material obtained from fine-needle biopsies, and can be generated from patients whose tissues are difficult to resect [46]. Therefore, this method provides more accurate results and more effective drug screening than genetically engineered mouse models and can be used in precision medicine [47]. In particular, organoids can be established in resected tissues before chemotherapy or after neoadjuvant chemotherapy to measure the response to anticancer drugs, which is useful for precision therapy, drug screening, and tumor biology research [48]. Recently, PDOs were generated using endoscopic ultrasound-guided fine-needle aspiration biopsies [46,49-52] and utilized in co-culture systems for drug screening [51,52]. Ascites or pleural fluid has also been used as a source to generate PDOs [53]. Pancreatic organoids can contribute considerably to the growing demand for translational research and their usage has expanded synergistically with multiomics, genome editing [54,55], microfluidic chips [56], bioprinting [57], and artificial intelligence [58].

Pancreatic organoids

Self-renewing organoids can be produced from primary pancreatic tissue, pancreatic tumor tissue, adult stem cells, ESCs, and iPSCs (Table 1). Primary pancreatic tissue has been used to generate self-renewing epithelial organoids with epithelial cells within tissues [12,13] and form organoids with neoplastic epithelial cells within pancreatic tumors [13,14]. Isolated cells or tissue fragments are typically placed in Matrigel and incubated in a medium containing growth factors and small molecules. The components of the medium vary depending on the species and organ [37]. After a few days, a 3D structure capable of continuous passage for months begins to form, and the organoids resemble cystic structures with a single polarized epithelial cell layer surrounding the central lumen. Tissue-derived epithelial organoids are genetically stable and used in a range of studies from basic processes to therapeutic applications, whereas tumor organoids are powerful tools for studying cancer in vitro because they capture the histological organization of the original tumor and retain its genomic environment, gene expression profile, and tumorigenic potential.

Since mature somatic cells were first efficiently reprogrammed into iPSCs [59], numerous protocols have been developed to differentiate iPSCs into specific cell types, leading to the 3D culture of PSC-derived cells and the generation of organoids [60]. The differentiation of PSCs into endodermal pancreatic progenitors precedes the formation of pancreatic epithelial organoids [14,61] and islet organoids [62]. In addition, protein C receptor+ (Procr+) endocrine precursor cells in the mouse pancreas have been used to generate islet organoids [63].

The first pancreatic organoids were generated in 2013 using mouse pancreatic tissue [12]. When pancreatic ducts from adult mice were isolated and placed in 3D culture, they exhibited ductal morphology and formed organoids after 2 days; these pancreatic ductal organoids were capable of self-renewal for over 40 weeks. Under these conditions, pancreatic ductal organoids can expand into cystic structures without endocrine compartments, and differentiate into pancreatic ductal and endocrine cells upon transplantation [12]. These culture conditions were extended to adult human pancreatic ductal organoids and mouse neoplastic tissues, and for the first time, primary and metastatic PDACs were established and cultured as tumor organoids [13]. These PDAC organoids were generated from resected tissue and biopsies obtained by fine-needle aspiration and showed disease stage-specific properties, after which they were transplanted into mice to monitor the pro-

Table 1. Established pancreatic organoids

	2013 [12]	2015 [13]	2015 [64]	2015 [14]	2016 [67]	2017 [61]	2018 [68]	2018 [69]	2020 [63]	2020 [62]	2021 [72]
Source	Mouse pancreatic duct	Adult human (normal tissue, tumor tissue) Mouse (tumor tissue)	Human and mouse fetal pancreatic progenitors	Human ESCs, PDAC	Acinar cells	Human PSC (ESC, iPSC)	Adult human pancreatic tissue biopsies without islets	Human PDAC fine needle aspiration and ascites biopsy	Procr+ endocrine precursor cells from adult mouse pancreas	iPSC	Human PSC (ESC, iPSC)
Organoid	Pancreatic ductal organoids	Human pancreatic ductal organoid, PDAC organoid	Pancreatic organoids	Pancreatic organoids, PDAC organoid	Acinar-derived organoids	Pancreatic organoids	Pancreatic ductal organoid	PDAC organoid	Pancreatic islet organoids	Islet like organoids	Pancreatic ductal organoid, acinar organoid
Special feature	Bi-potentiality after transplantation (duct cells, endocrine cells)		Differentiated into endocrine		Acinar-to-ductal metaplasia	Cystic fibrosis pancreas model	Tips of the budding structure	Co-culture with CAF, cancer subtype	Co-culture with endothelial cells	Type 1 diabetes model, immune evasion (PD-L1 overexpression)	KRAS-G12D, IPMN

PSC, pluripotent stem cell; PDAC, pancreatic ductal adenocarcinoma; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; CAF, cancer-associated fibroblast; IPMN, intraductal papillary mucinous neoplasm.

gression from early grade to invasive and metastatic tumors [13]. Mutations in the primary tumor are maintained and can be genetically manipulated, which is an advantage of PDAC organoids for modeling disease progression [13]. These 3D culture conditions have also been applied to organoids with human and mouse fetal pancreatic progenitors [64]. In these cases, the organoids exhibited pancreatic progenitor characteristics and could be continuously cultured with stimulation of R-spondin1, FGF10, and EGF. Upon the removal of EGF, proliferation was inhibited and endocrine gland differentiation was promoted [64].

After the establishment of the first pancreatic organoids, pancreatic epithelial and PDAC organoids were generated from PSC-derived pancreatic progenitors and PDACs, respectively [14]. Unlike organoids formed from primary tissues, pancreatic organoids derived from PSCs formed pancreatic exocrine structures containing carboxypeptidase A1 acinar and KRT19 ductal compartments. The change in organoid phenotype was induced by the expression of mutant KRAS or TP53, while PDAC organoids retained the characteristics of the primary tumors and patients. These organoids have been proposed as a PDAC model for precision therapy drug screening [14]. ESCs and iPSCs were differentiated into pancreatic progenitors [65,66] and cultured as pancreatic epithelial organoids under 3D culture conditions [61], and each organoid cell consisted mostly of acinar and ductal cells. The ductal cells of the PSC-derived pancreatic organoids exhibited carbonic anhydrase activity, while the acinar cells showed amylase, trypsin, and elastase activities, indicating normal pancreatic function. The organoids were transplanted into the pancreases of immunodeficient mice, where they were functionally engrafted via angiogenesis and resembled the human fetal pancreas [61]. The pancreatic organoid was proposed as a disease model of cystic fibrosis [61].

Pancreatic acinar cells were cultured with organoids under similar conditions to those for pancreatic ductal organoids using primary tissues [67]. After 8 to 10 days, the acinar-derived organoids adhered to the bottom and formed duct-like structures and five days after isolation, the acinar cells underwent acinar-to-ductal metaplasia, expressed the ductal marker CK19, and exhibited a cuboidal epithelial morphology [67]. A progenitor-like acinar cell subpopulation was identified in the pancreatic acinar cells that was highly proliferative and expressed the STMN1 marker. This subpopulation likely contributed to the pancreatic acinar organoid formation [67].

Organoids formed from primary tissues and tumors have been used in many studies. Pancreatic ductal organoids have been formed from adult human pancreatic tissue biopsies that lacked islets [68]. A cell subpopulation with high aldehyde dehydrogenase activity at the tip of the budding structure exhibited progenitor characteristics. These cells had gene expression profiles similar to those of human fetal pancreatic tissue and differentiated into endocrine cells in vitro, after which they were transplanted and differentiated into insulin-secreting cells [68]. Tumor organoids were generated by fine-needle aspiration and ascites biopsy from 39 patients with PDAC [69] and co-cultured with cancer-associated fibroblasts (CAF) to establish PDAC- and CAF-fused organoids [69,70]. The PDAC organoids were used to identify three tumor subtypes (Wnt-non-producing, Wnt-producing, and R-spondin-independent) based on niche factor dependence on Wnt and R-spondin, with decreased GATA6 expression as Wnt dependence decreased. The tumor organoids resembled the primary tumors and exhibited tumor-specific mutations [69]. Co-culturing PDAC and CAF has helped elucidate the interaction between the two and the characteristics and types of CAF [69,70]. In addition, PDAC organoids were treated with FOLIRINOX, an anti-PDAC drug combination, to form chemoresistant organoids through a similar process by which this resistance develops in patients. Therefore, chemoresistant PDAC organoids can be useful for elucidating the mechanisms of chemoresistance [71].

Organoids formed from PSCs are also useful in studies on cancer development and modeling of specific oncogenic mutations. The hPSC-derived pancreatic progenitor was induced to differentiate into pancreatic ductal and acinar organoids using two differentiation strategies [72]. Both organoid types exhibited cell type-specific functions by expressing the typical ductal markers SOX9 and carbonic anhydrase II or the acinar markers pancreatic transcription factor 1 and chymotrypsin C, respectively. When the acinar organoids were genetically engineered to express KRAS with a cancer-associated form, the G12D mutation, they exhibited ductal metaplasia in vitro and formed early PDACs in vivo [72]. Furthermore, the mutation caused the ductal organoids to form intraductal papillary mucinous neoplasm (IPMN)-like structures, resulting in a cell lineage-specific phenotype [72]. A pancreatic ductal organoid established using hPSCs-derived pancreatic progenitors showed oncogenic mutations with distinct morphological changes and molecular phenotypes [73]. The combination of oncogenic KRAS expression and CDKN2A loss caused the pancreatic ductal organoid to form PDAC-like lesions. Similarly, the mutant GNAS-expressing pancreatic ductal organoids formed IPMN-like lesions [73].

Efforts have been made to improve the culture of organoids. Human pancreatic ductal organoids that were generated from fresh and cryopreserved primary tissues using a chemically defined culture medium were greatly expanded in the long term [74]. The addition of TGFβ inhibitors, forskolin, and prostaglandin E2, combined with an increase in the concentration of RSPO1, generated organoids with an efficiency of over 90% and over 180 days of culture generated organoids with an efficiency of over 90% and over 180 days of culture. In addition, the pancreatic ductal organoids formed closely resembled the primary tissue. Similar results were obtained using a dextran-based hydrogel [74].

Pancreatic islet organoids

Finally, pancreatic islet organoids were generated by inducing the differentiation of iPSCs into endocrine progenitors, followed by 3D culture [62]. These organoids were enriched in endocrine cells, with 50% to 60% of cells expressing both insulin and β-cell markers, while single-cell transcriptome analysis identified β-, α-, δ-, and γ-cell populations [62]. When transplanted into diabetic NOD/SCID mice, the islet organoids rapidly improved blood glucose levels. Moreover, overexpression of the immune checkpoint protein PD-L1 protected the islet organoids from host immunity and improved blood glucose levels for 50 days after transplantation, even in immunocompetent mice. In addition, the PD-L1-expressing islet organoids treated with interferon-γ showed immune-evading properties and improved blood glucose levels [62].

Islet organoids have also been established from endocrine progenitors. Single-cell RNA sequencing was used to identify a new cell population expressing Procr in adult mouse islets [63]. Islet organoids were formed from $Procr^+$ pancreatic islet progenitors by co-culture with endothelial cells. This $Procr^+$ cell population generated β, α, δ, and PP cells to form functional islet organoids that could be maintained long-term and transplanted to control blood glucose levels in a type 1 diabetes mouse model [63]. Additionally an insulin-secreting organoid generated from the stomach was transplanted into a diabetic mouse model to achieve glucose homeostasis [75].

Recent PDAC application

The field of preclinical research on PDAC using pancreatic and PDAC organoids has expanded considerably in recent years, particularly in the areas of PDAC organoid modeling, PDAC pathogenesis, microenvironments, drug screening, and drug response predictions for precision therapy [76,77].

Studies of oncogenic gene mutations have suggested that KRAS mutations can cause PDAC. Pancreatic organoids have shown dysplasia due to the expression of a G12D mutation in KRAS, the loss of p53, or both and subsequently developed adenocarcinoma after transplantation in vivo [78]. In addition, the G12V mutation in KRAS caused tumor-like mutation-specific phenotypes in pancreatic organoids in culture and in vivo [14]. KRAS mutations initiate the tumor process as pancreatic intraepithelial neoplasia (panIN), which then progresses to pancreatic cancer [79]. Expression of the KRAS G12D mutant in organoids resulted in lumen-filling, growth arrest, and epithelial-to-mesenchymal transition features and led to differentiated PDAC after transplantation [73]. In addition, branched-chain amino acid metabolism is potentially associated with PDAC development, whereby branched-chain amino acid transaminase 2 can cause branched-chain amino acid catabolism, which enhances panIN formation in pancreatic organoids carrying KRAS mutations [80].

Although many PDAC classifications have been studied, two main subtypes have been established that differ in their response to treatment. The basal-like epithelial subtype is characterized by the expression of basal markers, such as cytokeratins and is associated with a poor prognosis, whereas the classical epithelial subtype expresses ductal markers such as GATA6 and has an improved prognosis [81]. Chemotherapy induces plasticity in PDAC cells by switching between the basal subtype and the classical subtype, which can be explained as cancer cells switching subtypes to adapt to chemotherapy. Tumor organoid subtypes differ in their dependence on changes in the tumor microenvironment. Various in vivo niches or cytokine treatments alter the transcriptome of PDAC cells based on external factors, indicating the plasticity of these cells [82-84]. These subtypes can be maintained and develop chemoresistance. After FOLFIRINOX treatment, the classic subtype was retained; however, marked differences were evident in the in vitro drug response [85]. These results indicate that drug adaptation may be achieved through various unknown mechanisms. The PDOs used in these studies were found to be optimal for longitudinal comparisons of the characteristics of the two epithelial subtypes of PDAC and their response to anticancer drugs, pre- and post-treatment. Therefore, these PDOs can become useful tools for precision therapy.

PDAC organoid studies on the tumor microenvironment have largely relied on human tumor tissue or cell line-derived organoids co-cultured with CAFs from patients [86]. These co-cultures have been used in many studies to establish CAF

types, niche factors, characteristics, and PDAC models [69,70,87,88] and elucidate the role of CAFs in generating the extracellular matrix and the mechanisms leading to anticancer drug resistance [89]. In vitro organoid models established through the co-culture of pancreatic tumor cells, stroma, and immune cells can act as important research tools for analyzing interactions between pancreatic cancer stroma and immune through the use of CAF types and infiltrating immune cells [87]. In addition, co-cultures of PDAC organoids and immune cells can be used to characterize tumor immune cells and study immunotherapies [90]. Recently, PDAC, iPSC-derived mesenchymal cells, and vascular endothelial cells were co-cultured to generate fused pancreatic cancer organoids that were divided into quiescent and proliferative cells according to their characteristics and anticancer drug response. These organoids could then be used for anticancer drug screening [91]. Furthermore, a vascularization model has been proposed to accurately reproduce the tumor microenvironment [92].

The microenvironment of organoid models is not homogeneous with fibers and cells. Different types of CAF exist, such as myofibroblastic CAFs that express α-smooth muscle actin and are found near tumor cells, and inflammatory CAFs that secrete IL-6 and IL-11 and are distant from tumor cells [70]. These types can transform into one another and hypoxia has been shown to promote the transformation of inflammatory CAFs by cytokines secreted by the tumors [93].

The tumor microenvironments have also been subtyped. A "reactive" sub-tumor microenvironment with an abundant population of activated CAFs is associated with tumor progression and a greater immune response, while a "deserted" sub-tumor microenvironment has fewer activated CAFs and is associated with differentiated CAFs, tumor response to treatment, and a lower immune response [94]. Changes to the type of CAF by the culture conditions of the organoids will alter the microenvironment, which in turn will affect the tumor response to treatment. IL-1 induces an inflammatory CAF state [95], and the transcription factor Prrx1 activates CAF to induce plasticity [96]. Deletion of the latter causes tumor differentiation, disrupted tumor dissemination, and an epithelial-to-mesenchymal phenotype in the CAF that is indicative of gemcitabine resistance. Modulating these factors may improve the treatment response. Furthermore, CAF in the tumor stroma has been reported to induce an epithelial-to-mesenchymal transition that supports chemoresistance in PDAC [97] and induces platinum resistance via extracellular vesicles [98]. The tumor microenvironment may also cause an increase in tumor invasion [99]. In addition, CAFs are involved in acinar-to-ductal cell transdifferentiation and may induce pancreatic cancer through LAMa5-ITGA4 [100].

Screening for anticancer drugs is a priority for precision therapy in PDAC treatment [101-105]. The in vitro sensitivity measurements of 76 therapeutic agents using PDOs allowed for the identification and proposal of new therapeutic agents [101], and drug screening in particular has been suggested to treat chemotherapy-resistant pancreatic cancer [102,103]. The FDA-approved compounds included a novel anticancer drug with therapeutic effects on PDAC [104] and a molecular predictor for the prognosis of patients treated with adjuvant gemcitabine [106]. A chromatin accessibility atlas was constructed from the PDO of patients with PDAC, and chromatin accessibility signatures were reported to be associated with chemotherapy sensitivity [107]. The anticancer effects of perhexiline maleate on PDAC organoids and PDAC organoids with KRAS G12D mutant was discovered, and the relevance of KRAS mutations to the cholesterol synthesis pathway was reported [108].

The clinical utility of PDOs can be maximized if the response to anticancer drugs by patients with PDAC can be predicted. PDOs of PDAC patients have been constructed, tested for drug sensitivity, and correlated with their clinical responses [45,48,109,110], which helps in the selection of anticancer drugs and the prediction of patient outcomes. In addition, organoids derived from circulating tumor cells have been correlated with drug sensitivity and clinical responses to treatment [111] through studies in the laboratory and in the clinic. Somatic mutation and copy number variants data from PDOs were used to improve the quality of clinically meaningful information, suggesting that PDOs can be used as ex vivo models to facilitate precise cancer treatment [109]. Sensitivity to anticancer drugs was measured in relation to changes in tumor markers and images, thereby allowing for prospective prediction of chemotherapy responses. A previous study reported that a rapid PDO drug screen performed within 7 days of tissue collection correlated with the chemotherapy response of the PDO and the pathological response of the patient [48]. Generating PDOs using tissues before and after neoadjuvant therapy allows for serial measurements of chemotherapy sensitivity to identify changes that can then be applied to precision therapy. PDAC organoids from patients treated with or without FOLFIRINOX were used as single agents or in combination with FOLFIRINOX, and the results showed significant drug resistance in PDAC organoids after FOLFIRINOX treatment [112]. Organoids from the FOLFIRINOX-treated patients proved useful for predicting treatment response and an-

alyzing anticancer drug resistance and its mechanisms.

In addition to PDAC, pancreatic organoids have been used to study several other diseases, such as diabetes, in which the replacement of damaged islets, post-transplant survival, immune evasion, implantation location, and 3D bioprinting are major challenges [113,114]. Recently, studies have established islet organoids from iPSCs and Procr^{+} pancreatic islet progenitors [62,63] as well as a model to evade immunity after transplantation [62]. A liver-islet axis simulation using a microfluidic multiorganoid system has been developed to study type 2 diabetes [115]. In addition, a model that co-cultured islets and amniotic fluid epithelial cells, known to exert protective effects, improved transplantation success [116], and genes promoting angiogenesis and cell function were upregulated to enhance engraftment after transplantation [117]. In the field of cystic fibrosis, the main challenges are the establishment of disease models and modulator studies [118], and pancreatic ductal organoids have been proposed as a suitable models for studying exocrine ion secretion in relation to this disease [119,120]. A pancreatic organoid model of cystic fibrosis was established using iPSCs from cystic fibrosis patients [61], while another model was developed using a pancreas-on-a-chip composed of pancreatic duct epithelial cells and islet cells [121].

Perspectives

Despite the development and diverse application of pancreatic organoids, various challenges remain [122]. Standardized procedures for organoid generation and culture have not yet been established, and standardization is needed to reduce the variation among the organoids. Furthermore, comparisons to complex living organisms are difficult due to the challenges in reproducing the proper cellular composition, extracellular stroma, supportive tissues, immune environment, vascular connections, and neural control. Most organoids are established in Matrigel for 3D culture, which can be disadvantageous for clinical applications because of the presence of unknown components and the requirement for various growth factors and small molecules in the culture medium. In terms of clinical application, animal-derived materials are used in the generation and culture process, and it is difficult to produce sufficient tissue for transplantation and immune rejection is often induced during transplantation. In cancer research, tumor organoids are a mixture of closely related tumor and normal cells. Organoid models are becoming more sophisticated by incorporating co-cultures, 3D scaffolds, biochips and alternatives to Matrigel, and gene-editing technologies are increasingly adopted to genetically modify organoids. Furthermore, bioreactors are generating larger and higher volumes of organoids to address some of the challenges. Pancreatic organoids derived from primary tissues and stem cells are mainly used for studying pancreatic physiology, type 1 diabetes, and cystic fibrosis, while organoids derived from cancer tissues of pancreatic cancer patients are used to study PDAC and endocrine tumors and establish anticancer treatment strategies through drug screening. The use of these organoids is expected to expand to other research fields and clinical applications in the near future.

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Ethics approval

Not applicable.

Conflict of interest

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