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Modeling pancreatic cancer with organoids

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Abstract

Pancreatic ductal adenocarcinoma (PDA) is a highly lethal malignancy for which new treatment and diagnostic approaches are urgently needed. In order for such breakthroughs to be discovered, researchers require systems that accurately model the development and biology of PDA. While cell lines, genetically engineered murine models, and xenografts have all led to valuable clinical insights, organotypic culture models have emerged as tractable systems to recapitulate the complex three-dimensional organization of PDA. Recently, multiple methods for modeling PDA using organoids have been reported. This review aims to summarize these organoid methods in the context of other PDA models. While each model system has unique benefits and drawbacks, ultimately, organoids hold special promise for the development of personalized medicine approaches.

The Need for Accurate Model Systems of Pancreatic Cancer

Pancreatic cancer is an extremely lethal malignancy, with a 5-year survival rate of less than 7% [1]. This malignancy is currently the fourth leading cause of cancer deaths in the United States [1] and is projected to surpass colon and breast cancers by 2030 [2]. Pancreatic ductal adenocarcinoma (PDA), the most common form of pancreatic cancer, is often diagnosed too late for effective therapeutic intervention to succeed, and only 10–15% of patients diagnosed with PDA are eligible for surgery, the only potentially curative option [3]. Thus, new approaches to diagnose and treat PDA are urgently needed.

In order for clinical progress to be made, researchers need accurate and tractable model systems for studying this malignancy. At first glance, it would seem that the best way to study PDA would be to use human tumor tissue. However, the use of such tissue has limitations as a model. Human tumors are often diagnosed late in tumor evolution, providing a snapshot of a late stage of tumorigenesis, and offering little utility to the study of tumor

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development. In addition, as only a small number of PDA patients are eligible for surgical intervention, the amount of human tumor tissue available for research is limited. Therefore, methods to culture and/or propagate tumor tissue are valuable as they offer researchers a way to probe cellular pathways involved in tumorigenesis or to ascertain the effects of therapeutics. Traditional model systems such as genetically engineered mice, cell lines, and xenografts have all led to powerful insights into PDA tumorigenesis. More recently, researchers have turned to three-dimensional (3D), organotypic culture methods, which hold great promise to circumvent some of the limitations of traditional models. Advances in the field of regenerative medicine have led to the development of organoids as 3D models. Recently, these developments came together, with the report of four new methods for modeling pancreatic cancer using organoids [4–7] (Figure 1). Here, we discuss traditional methods for modeling PDA as well as these new organoid approaches. We also describe the challenges and opportunities for using PDA organoids for clinical care.

Monolayer Cell Lines

Monolayer neoplastic cell lines have been a mainstay of cancer research. The first human cancer cell line was generated from the cervical tumor of Henrietta Lacks and therefore named HeLa cells [8]. Eleven years later, the first PDA cell line was reported [9]. Since that time, numerous cell lines have been derived and represent tractable models for many different types of human cancer. Once adapted to growth in serum-containing media in tissue culture dishes, cancer cell lines are generally easy to culture, and can be passaged, cryopreserved and manipulated both genetically and chemically.

While the use of monolayer cell lines in the study of cancer biology has led to numerous insights, cell lines suffer from several limitations. Many tumors, especially slower growing, non-metastatic lesions, fail to generate cell lines, with prostate cancer being a prime example [10]. For pancreatic cancer, the efficiency of generating cell lines from a resected primary tumor is lower than the efficiency of generating 3D cultures [5]. Moreover, since most cell lines are derived from a resected tumor specimen, and most PDA patients are ineligible for surgical resection, PDA cell lines can only be generated for a small subset of patients and therefore do not represent the diversity found across PDA tumors. In addition, neoplastic cell lines fail to recapitulate key features of tumor biology, such as interactions with stromal cells and extracellular matrix (ECM). Furthermore, neoplastic cellular polarity, cell-cell contact, and 3D organization are difficult to model when cells are cultured as monolayers [11–13].

The adaptation of neoplastic cells to monolayer tissue culture conditions has been shown to fundamentally alter cells, selecting for clones that no longer model their primary counterparts [14]. Culturing cells as monolayers in the presence of serum has been shown to promote the loss of p53 function and to drive genomic instability [5, 14, 15]. Numerous studies have identified gene expression differences between monolayer cell lines and their corresponding primary tumors [12, 15–18], including comparisons of pancreatic cancer cell lines to primary PDA [18, 19]. These differences remain even when the presence of stromal cells in the primary tumor is taken into account [12, 18]. Thus, while there are many advantages to working with cancer cell lines, they may not accurately model many aspects of tumor biology.

Reprogrammed Monolayer Cultures

As an alternative to standard, monolayer PDA cell lines, two recent publications report methods that involve the addition of factors to support monolayer cultures. Kenneth Zaret and colleagues isolated epithelial cells from a resected human PDA tumor and then induced expression of the Oct4, Sox2, Klf4, and c-Myc transcription factors to reprogram the cancer cells [20]. These reprogrammed cells required sustained expression of the transcription factors to remain undifferentiated and were therefore called “induced pluripotent stem (iPS)-like” cells to differentiate them from other iPS cells, which do not require sustained expression of the reprogramming transcription factors. When the PDA-derived iPS-like cells were transplanted subcutaneously into immunocompromised mice, they gave rise to structures that resembled pancreatic intraepithelial neoplasms (PanINs), pancreatic lesions believed to be the precursors of PDA [20]. These results suggest that cellular reprogramming may partially suppress the cancer phenotype, and this technique allows for a model of the earliest stages of tumorigenesis. To date, this method has only been reported to successfully allow culture of one human tumor, and the relatively low efficiency of colony formation suggests that clonal selection may be a potential limitation of this technique. However, if the robustness of the method can be demonstrated, it could be a useful approach for cultivating tumor cells from many patients.

Richard Schlegel and colleagues have developed an alternative approach to culturing primary human tumor cells in which the cells are propagated on top of a confluent layer of irradiated fibroblasts in the presence of an inhibitor of Rho-associated, coiled-coil-containing protein kinase (ROCK), generating cultures which the authors call “conditionally reprogrammed cells” [21]. ROCK signals downstream of the Rho GTPase pathway, and is involved in focal adhesion formation, cell migration, stress fiber formation and regulation of apoptosis [22]. Inhibition of ROCK promotes cell survival following dissociation [23]. How the irradiated fibroblasts support growth is not entirely clear, but it likely involves secreted factors, since conditioned media from irradiated fibroblasts can also promote the growth and survival of the tumor cells [21]. While these approaches hold promise as new PDA models, the lack of 3D architecture in these cultures remains a limitation. In addition, whether these culture conditions select for a subset of tumor cells remains unclear.

Genetically Engineered Mouse Models

The development of technologies to genetically engineer mice with specific mutations has led to the generation of numerous murine models of PDA. (For a review, see [24]). Many of these models use pancreas-specific *Cre* drivers such as *Pdx1-* or *P48-Cre*, to activate oncogenic alleles of *Kras*, a gene mutated in more than 90% of human PDA tumors [3]. Tumors arise spontaneously in these mice, allowing early tumorigenesis to be studied. In addition, tumors develop in 3D, and contain the dense desmoplasia and poor vasculature characteristic of human PDA [25].

Genetically engineered mouse models offer an advantage over many of the other methods to study PDA in that these models have native interactions between the neoplastic and stromal cells. Thus, these models allow researchers to study interactions between tumor cells and the native immune system and to explore the effects of immunotherapies [26]. In addition

studies using genetically engineered mice have revealed the complex interplay between PDA and stromal cells, showing that cancer associated fibroblasts can play a tumor supportive [27, 28] or tumor suppressive role [29, 30], depending on the context. Murine models have led to numerous clinical insights into PDA, such as the discovery that albumin-coated paclitaxel could augment the potency of gemcitabine by decreasing levels of cytidine deaminase, an enzyme responsible for gemcitabine metabolism [31].

Despite the benefits of using engineered mice to study PDA, rapid experimental interrogation is difficult with these models. Mouse models are time-consuming and expensive to breed, and the engineering of additional mutations requires considerable effort. Furthermore, there have been discoveries in murine models that have not been reproduced in the human setting [25, 30]. These may have been the result of interspecies differences, such as differences in drug metabolism [32], immune function [33], and telomerase activity [34] between mice and humans. In addition, in some murine PDA models, the oncogenic alleles are expressed during development, and murine tumors are smaller and arise from fewer cell divisions than human tumors [35]. Finally, murine models of PDA tend to have fewer mutations and less genetic complexity than human tumors [36]. Taken together, murine models are powerful tools for interrogating PDA biology, but scientists planning or interpreting studies involving murine models should be cognizant of these limitations.

Xenograft Approaches

Alternative methods to model PDA involve the engraftment of human cell lines (cell-line-derived xenografts, or CDXs) or human tumor fragments (patient-derived xenografts or PDXs) into immunocompromised mice. CDXs are generated from cultured cell lines and therefore suffer from many of the limitations described for monolayer cell lines, including selection during culturing. In addition, while some have reported that xenograft tumors generated by subcutaneous injection of cancer cell lines share histological characteristics with their corresponding primary tumors [37], others have found that these tumors fail to recapitulate primary tumor histology [14, 38]. Furthermore, activity of therapeutic agents on CDX models was found to have poor correlation with activity in human patients [39, 40]. This low correlation held true for numerous studies using PDA xenografts (reviewed more extensively in [41]), and may be attributable to the dense desmoplastic stroma and low blood vessel density found in human PDA, which are not recapitulated in CDX’s of this malignancy [25].

In contrast to CDXs, PDXs retain many of the characteristics of their corresponding primary tumors, including tissue architecture and genomic alterations [42–45]. While subcutaneous engraftment is commonly used to establish PDXs from human PDA, resemblance to primary tumor was best when PDXs were established through transplantation into the pancreas (orthotopically) [46, 47]. These results suggest that while orthotopic transplants are more cumbersome to perform, they may generate more reliable data. Successful engraftment of PDA tumors was shown to be significantly correlated with shorter survival [48], suggesting that highly aggressive tumors are more amenable to PDX generation. PDXs have been used to find mechanisms of resistance to targeted therapies and biomarkers to predict therapeutic response [44]. However, following engraftment, PDXs derived from PDA become infiltrated

with murine stroma [43], and most human stroma is replaced with murine cells long before the fourth or fifth passage which is when PDXs are most likely to be used for clinical testing [42]. The mismatch between human tumor cells and mouse stroma should be considered when evaluating studies involving PDXs.

Overall, studies have found that clinical response of PDXs to therapeutics is correlated with response in patients (reviewed in [42]). However, only limited studies have been done to look at the clinical response of PDXs generated from PDA patients. Manuel Hidalgo and colleagues compared the clinical response of 23 patients and their corresponding PDXs when treated with gemcitabine, and found that in 7 of the 23 pairs, the sensitivity of the PDXs to gemcitabine correlated with a longer median time to progression in the patient [48]. A separate study from this group subjected 4 PDA-derived PDXs to 63 different therapies in 232 combinations to determine the most effective agents against each PDX [49]. While 3 of the 4 PDXs were most sensitive to gemcitabine, which is already a standard of care for PDA, 1 PDX showed sensitivity to mitomycin C and cisplatin. When the patient matching this PDX progressed on gemcitabine, he was treated with mitomycin C and later with cisplatin, and was reported to be disease-free 50 months after his initial diagnosis [49]. These results suggest that PDXs may have predictive value in the clinic, but further studies are needed to determine how well they correlate with the response of PDA patients and to better understand the reasons why responses of some PDXs fail to correlate with those of patients.

While initial studies using PDX models are promising, this model has a number of limitations that may prevent its widespread use. Relatively large amounts of resected tissue or biopsy material are needed to generate PDX models, limiting the applicability of this model to most PDA patients. Moreover, PDXs take 4–8 months to produce and require lengthy passaging to generate experimental cohorts [50]. The generation and maintenance of large numbers of immunocompromised mice to passage the PDXs can be costly. Finally, the use of immunocompromised mice limits the use of PDXs to study immunotherapies. Nonetheless, the PDX method allows for the propagation and study of many types of tumors in an *in vivo* setting.

Three Dimensional Models of Pancreatic Tumorigenesis

The limitations in the systems available to study pancreatic tumorigenesis prompted researchers to find novel ways to model PDA. To that end, 3D culture methods hold promise as systems to better mimic the biology found *in vivo*. A key goal of 3D culture methods is to prevent cells from attaching to the bottom of the culture dish, either by keeping cells in suspension or by culturing cells in the presence of a matrix such as collagen or Matrigel (Box 1). 3D methods to culture pancreas cells are hardly a new phenomenon. Rather, they have emerged as part of a larger movement over the last century to be able to culture and study tissues *ex vivo*.

Box 1

3D Culture Matrices

3D culture methods typically involve embedding cells inside of or culturing cells on top of a matrix. Substances such as Matrigel, hyaluronic acid, laminin, collagen, polyethylene glycol hydrogels, photoactivatable hydrogels, and peptide hydrogels have all been used as matrices for 3D culture [111, 112]. These substances provide a physical barrier, preventing cells from interacting with and attaching to the culture dish. In addition, the matrix acts as a physical structure with which cells can interact. Depending on the matrix material, this structure provides the cells with biomechanical and sometimes biochemical cues that mimic the environment of the ECM in vivo [112]. Collagen and Matrigel are the two most common matrices used for 3D culture of pancreas cells.

Collagen

There are at least 26 distinct types of collagen, of which types I and IV are most often used for culture. Type I collagen is the most abundant collagen in the human body, and is found in tendon, bone, skin, and other interstitial connective tissues [113]. This collagen can be purchased either as recombinant protein or purified from rat tail. Type IV collagen is the primary component of the basement membrane of normal tissue, including pancreatic epithelium [113–115]. PDA progression is associated with breakdown of the basement membrane architecture and with increased production of type I collagen as part of the desmoplastic reaction [116, 117].

Matrigel

Matrigel is a commercial name for a basement membrane extract that is purified from murine Engelbreth-Holm-Swarm (EHS) tumors [118]. Matrigel contains high levels of type IV collagen as well as laminin and enactin/nidogen [118]. In addition, more than 1800 proteins have been identified in Matrigel, including numerous growth factors such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), transforming growth factor beta (TGF-β), and platelet-derived growth factor (PDGF) [119]. For researchers wishing to control growth factor levels in their cultures, a growth factor-reduced preparation of Matrigel is available [120], although levels of TGF-β are not much reduced in this preparation, which may impact the growth of some cell types [119]. When cultured in Matrigel, numerous cell types, including pancreatic cells, have been shown to take on a polarized morphology and to have physiologic responses not seen in cells cultured as monolayers [118].

The Development of Three-Dimensional Approaches to Culturing Pancreas Cells

Researchers have been attempting to culture the pancreas outside the body as far back as 1938, when Carrel and Lindberg were able to keep a cat pancreas alive in culture for 4 days using a perfusion pump [51]. By the 1980s, numerous methods for culturing the pancreas as organ explants had been reported and researchers were starting to develop methods for culturing isolated pancreas cells in 3D [52]. One early study found that primary rat

pancreatic duct cells cultured in agarose formed closed lumina and could be maintained in culture up to 6 weeks [53]. At this time, primary culture methods were employed to aid in the study of pancreatic endocrine cells. For instance, when cultured in the presence of collagen, dissociated rat pancreas cells were reported to associate into “islet-like organoids” [54]. Human pancreas cells embedded in Matrigel or in rat tail collagen were found to proliferate and form cysts that expressed pancreatic ductal markers as well as structures resembling endocrine islets [55]. While these early methods did not allow for the passage of pancreatic cells in culture, they did provide valuable insights into pancreatic development and endocrine function.

Building off the successes of early explant cultures, researchers have developed methods for organotypic culture of pancreatic tissue slices. Speier and colleagues were able to successfully culture murine pancreas tissue slices embedded in agarose for up to 7 days [56]. Similar methods were used to culture human normal pancreas tissue and resected PDA tumor specimens as organotypic slice cultures, which were viable for up to 6 days and amenable to transduction with adenoviral vectors [57]. By using a precision vibratome and slicing tissue immediately following surgical resection, Loda and colleagues were able to culture 300–500 μM slices of colon, lung, and prostate carcinomas without needing to embed in agarose [58]. This technique was used to culture resected human PDA tumors and to evaluate the effects of mitogen-activated kinase (MEK) and phosphoinositide 3-kinase (PI3K) inhibitors [59]. Organotypic slice cultures retain stromal cells and preserve tumor-stroma interactions, which could be a major benefit to these cultures. However, depending on the size of the slice, diffusion of compounds or oxygen may be limited in these cultures. In addition, organotypic slice cultures can only be maintained for up to a week and do not rapidly proliferate in culture, so the amount of material that can be generated from a single patient is limited.

Methods have also been developed to allow for limited culture of partially dissociated primary normal and neoplastic pancreas tissue, leading to advances in our understanding of pancreatic tumorigenesis. For instance, the Rustgi Laboratory developed a method to culture pancreatic ducts for up to 10 months embedded in collagen [60]. The Bar-Sagi Laboratory developed methods for isolating and culturing primary murine pancreatic duct cells on top of a laminin-coated coverslip, embedded in high-concentration of Matrigel, or as an overlay in low-concentration Matrigel on top of a Matrigel bed [61]. By isolating pancreatic duct cells from a mouse with the $Kras^{LSL\text{-}G12D}$ allele and exposing the cells to Cre, the researchers were able to study how oncogenic Kras prevents cellular senescence [62]. Immortalized human duct cell lines embedded in Matrigel formed invasive, disorganized structures only in the presence of the $Kras^{G12D}$ oncogene, a finding that was only revealed by 3D culture [63]. Murine tumor cells or human PDA cell lines cultured in Matrigel on top of a bed of agar could be treated with chemotherapies and chemical inhibitors to assay for drug sensitivity [64].

In addition to these primary culture systems, methods to culture human PDA monolayer cell lines in 3D were developed. In one early study, cells from a PDA cell line were cultured under continuous agitation, which prompted the cells to self-organize into polarized spherical structures called “spheroids” [65]. Since then, numerous methods have been

reported. Some generate PDA spheroids by preventing cellular attachment through the use of special culture devices [66], low-attachment or agarose coatings [67–69], mechanical stirring [70], or by only passaging floating spheres within the culture well [71]. Others culture cells on top of or embedded inside of matrices such as fibronectin, methylcellulose, type I collagen, or Matrigel (Box 1) [11, 72–75]. A recent study reported the culture of a human PDA cell line inside synthetic scaffolds made of biocompatible polymers [76]. Finally, methods to co-culture PDA cells with stromal cells in 3D have also been described [77, 78]. Unlike the monolayer cell lines from which they are derived, spheroid cultures have been shown to take on characteristics more similar to those of cells *in vivo*, including the production of ECM, increased resistance to therapies, and the appearance of polarized cell junctions [68, 69, 75]. However, as described in the previous section, the use of monolayer cell lines as a starting point limits the *in vivo* relevance of these approaches.

While some use spheroid cultures to mimic 3D interactions found *in vivo*, others use spheroids as an assay for cells capable of self-renewal. In the latter case, cells must be seeded for spheroid formation at a low density, ensuring that the outgrowing spheroids were derived from propagating cells [79]. Such methods have been used to enrich for cells in the pancreas with the properties of adult stem cells, including the capacity to self-renew and the ability to differentiate into multiple lineages [80, 81]. Similarly, spheroids derived from PDA cell lines seeded at a low density have been used to enrich for cells with the characteristics of cancer stem cells [79]. Such spheroids have been shown to have enhanced capacity to form tumors following transplant and enrichment of known stem cell markers such as CD44 and CD133 [69, 71, 82]. However, the use of spheroid models as an assay for cancer stem cells should be interpreted carefully: while spheroids assay for the capacity of an isolated set of cells to self-renew under the culture conditions, it remains unclear how such cells correspond to tumor cells *in vivo* [83]. In addition, variation in spheroid methods, the propensity for spheroids to aggregate in culture, and the potential for quiescent tumor stem cells not to proliferate under spheroid conditions all may influence the reliability of these assays [83]. Nonetheless, the clonogenic sphere-forming assay remains a powerful technique to enrich for cells with stem-like properties.

Organoid Cultures

As methods for culturing PDA cells in 3D were increasing in popularity, researchers in other fields were working out methods to culture primary normal tissue and later, primary tumor cells as “organoids.” Organoid models have been described for the stomach [84–86], small intestine [87–89], colon [87, 89], liver [90, 91], and mammary gland [92], as well as numerous other tissues [93]. In addition, tumor organoid models have been developed for breast [92], colon [89], and prostate [10] cancers. More recently, pancreas and pancreatic cancer organoids have been developed [4–7, 94, 95].

Currently, no standard definition of “organoid” exists. The term “organoid” literally means organ-like, reflecting the ability of organoid culture conditions to prompt cells to self-organize into structures mimicking the architecture of the organ from which they were derived. While some have suggested that the term “organoid” should be reserved for cultures in which more than one cell type is present, as in an intact organ [4], this definition has not

been universally adopted. Nonetheless, different organoid models generally share a couple of characteristics. As with many other 3D culture methods, organoids are cultured on top of or embedded inside of a matrix (Box 1). In contrast to organ explant or organotypic slice cultures, where a large piece of tissue is cultured as an intact unit, organoids are generated from smaller multicellular units (such as enzymatically or mechanically dissociated tissue) or from single cells. Organoids share many similarities with spheroid cultures, and the terms have often been used interchangeably in the literature. For clarity, we suggest that 3D cultures that are generated from cells initially cultured as monolayers fall under the purview of "spheroid" cultures. In contrast, the term "organoid" should be reserved for 3D cultures that are generated from dissociated primary tissue or from embryonic stem or iPS cells differentiated *in vitro*, and are propagated using a matrix and a media that promotes cell proliferation.

Non-neoplastic organoids are thought to arise from adult stem cells, and therefore should be capable of self-renewal and differentiation [96]. Consistent with this idea, flow-sorted pancreatic duct cells that stained positively for known progenitor/stem cell markers were more efficient at forming organoids than the general pancreatic duct cell population and much more efficient than progenitor-depleted duct cells [97]. The capacity for differentiation can be determined by transplanting the organoid into a mouse and demonstrating that the organoid can differentiate into structures resembling the tissue from which the organoid was derived. However, not all organoid systems have demonstrated both self-renewal and differentiation capabilities. In addition, whether tumor-derived organoids result from the outgrowth of a cancer stem cell population remains unclear.

The laboratories of Anne Grapin-Botton and Hans Clevers have both developed methods for culturing normal murine pancreata in Matrigel as organoids [94, 95]. Grapin-Botton and colleagues cultured murine embryonic pancreas cells inside Matrigel in order to study pancreatic duct development [94]. These embryonic pancreas progenitor organoids proliferated in culture and underwent branching morphogenesis. In contrast, the Clevers group, building on their prior work [86, 88, 89, 91], developed methods to propagate adult murine pancreatic duct cells as organoids [95]. When embedded in serum-free Matrigel and provided a defined, cocktail of growth factors, including the Lgr5 agonist R-spondin I to stimulate Wnt signaling, pancreatic duct cells formed proliferating, cystic spheres. Flow-sorting experiments have demonstrated that these conditions allow the propagation of pancreatic duct but not acinar or islet cells [97]. While these organoids retained ductal characteristics in culture, they could form both duct-like, Cytokeratin 19-positive structures as well as endocrine-like, Insulin-positive cells following transplantation into the mouse renal capsule, demonstrating that cells cultured with this method are bipotent [95].

In collaboration with the Tuveson Laboratory, the Clevers group extended this work to the culture of neoplastic pancreas cells [5]. Cells taken from PDA tumors and metastases that formed in genetically engineered mouse models could be cultured under identical conditions to normal pancreatic duct cells. Cells isolated from PanIN lesions that arise in genetically engineered mouse models could also be cultured as organoids. These organoids can be cryopreserved and passaged indefinitely, and are amenable to genetic, transcriptomic,

proteomic, and biochemical analyses. Thus, this system is ideal for probing changes involved at each stage in tumorigenesis.

With some modifications to the culture media, including the addition of Wnt3a, normal and neoplastic human organoids could be cultured. As with the murine organoids, human organoids proliferated readily under these conditions and could survive cryopreservation. While under current culture conditions, human normal organoids could only be cultured for 20–25 passages, human PDA organoids could be passaged indefinitely. Unlike the murine organoids, human PDA organoids took on a range of morphologies, often proliferating as filled spheres. This method worked well even with low numbers of starting cells, allowing for the generation of human PDA organoids from fine needle aspirate (FNA) biopsies and dramatically expanding the types of tumors capable of being studied in the laboratory. Like the murine organoids, organoids generating using this method are amenable to genetic and biochemical analysis: a recent study found that human patient-derived PDA organoids were sensitive to inhibition of MAPK-interacting protein kinase (MNK) with CGP57380 [98], showing that organoids can be used to study drug sensitivity.

In contrast to transplants of monolayer cell lines which rapidly form aggressive adenocarcinomas [25], orthotopic transplants of these murine or human PDA organoids initially give rise to structures resembling PanIN which only later progress to frank PDA resembling the tumor of origin [5]. A number of reasons could explain this transplant phenotype. The organoid culture system may “reprogram” more aggressive cancer cells to a less aggressive state. Alternatively, organoid culture may select for less aggressive cancer cells more likely to give rise to PanIN when transplanted. Monolayer cultures may also select for more aggressive cancer cells, which can readily form tumors following transplantation. It is also possible that less aggressive subclones present in the organoid cultures or normal cells present in the host mouse have a “neighbor suppressive” effect on PDA formation initially after transplant of the organoids. In line with this idea, *in vitro* studies using monolayer cultures have shown that fibroblasts can inhibit the growth of transformed cells [99], although the role of neighbor suppression in PDA remains unclear. More studies are needed to determine the biological basis for this transplant phenotype, and the relatively long time (2–8 months depending on the organoid culture) that transplanted tumors take to form frank PDA may be a drawback to this system. Nonetheless, the ability of PDA organoids to form PanIN-like structures following transplant can be exploited to study early pancreas cancer, which may aid in the discovery of new diagnostic tools. In addition, murine organoids derived from normal duct cells as well as PanINs, PDAs, and metastases provide a progression model to examine changes associated with each stage of pancreatic tumorigenesis.

Melissa Skala’s Laboratory recently described a similar method for culturing murine and human PDA organoids in Matrigel [7]. PDA tumors were isolated from *Ptf1a*$^{Cre/+}$; *Kras*$^{LSL\text{-}G12D/+}$; *Tgfbr2*$^{fl/fl}$ mice, digested, and embedded in a 50%/50% mix of Matrigel and serum-containing culture media. Under these conditions, both fibroblasts and cytokeratin-positive tumor cells were able to propagate, with the latter propagating as both spherical and asymmetric organoids. Organoids cultured under these conditions were amenable to optical metabolic imaging (OMI), which can be used to quantify levels of

NADPH and FAD as a proxy for cellular redox state and overall cell health. OMI was used to probe the response of murine PDA organoids to gemcitabine as well as to inhibitors of Janus kinase (JAK, AZD1480), MEK (AZD6244), or PI3K (XL147). Using this method, the authors were also able to generate organoids from a resected human PDA tumor [7]. Unlike the murine organoids, the human PDA organoids took on many morphologies. OMI revealed that the human PDA organoids were relatively insensitive to the JAK inhibitor, but showed some sensitivity to gemcitabine, or to the combination of gemcitabine with the JAK inhibitor. While relatively little characterization of these organoids has been reported, and the significance of the multiple organoid morphologies remains unclear, the OMI method may be of interest for those wishing to perform drug screens in 3D.

Senthil Muthuswamy and colleagues recently reported a third method for generating pancreatic organoid cultures [6]. Human pluripotent stem cells were converted to pancreatic progenitor cells *in vitro*, and cultured as an overlay in media on top of a Matrigel bed. Under these conditions, the progenitors organized into cystic spheres which secreted an outer layer of basement membrane and maintained gene expression patterns similar to those of pancreatic progenitor cells. Addition of exogenous ligands to stimulate Wnt signaling was not needed to maintain these cultures. Consistent with their progenitor state, these organoids were able to form both acinar and ductal cells when exposed to differentiation signals.

The Muthuswamy group extended this method to generate organoid cultures from surgically resected human PDA tumors with high efficiency [6]. Human PDA organoids showed dysplastic morphologies and often propagated as filled spheres. Organoids could be passaged and survived cryopreservation. Organoids subcutaneously transplanted into mice formed frank adenocarcinomas that resembled their tumors of origin. Intriguingly, a single culture of human PDA organoids could show multiple diverse histologies, reminiscent of the multiple histologies seen in the primary tumor, suggesting that this culture system may be capturing the intratumoral heterogeneity found in primary tumors. It is not clear whether untransformed human pancreatic tissue can be propagated using this organoid technique, which may limit experiments requiring an untransformed control generated under the same conditions. However, these organoids were amenable to small-scale therapeutic screening: the authors tested the growth of organoids in the presence of epigenetic pathway inhibitors and identified a subset of human organoids sensitive to inhibition of Enhancer of zeste homolog 2 (EZH2) [6]. The potential to use patient-derived organoids for therapeutic screening could have great clinical benefit.

Finally, Calvin Kuo and colleagues developed a distinct method for culturing pancreas cells as organoids [4], which built off their prior work culturing murine intestinal organoids [87]. The authors use an “air-liquid interface” (ALI) method in which embryonic tissue fragments are cultured in a type I collagen gel that rests on a permeable substrate with media underneath, allowing nutrients to diffuse from the bottom. The top of the culture is exposed to air, thus allowing cells to receive higher levels of oxygen than would be encountered in traditional culture methods and preventing hypoxic death. When grown as ALI cultures, murine neonatal pancreatic tissue formed cystic organoids consisting mainly of E-cadherin- and Pdx1-positive ductal epithelial cells surrounded by alpha-SMA-positive stromal cells

[4]. These cultures did not require exogenous growth factor supplementation and were viable for up to 50 days, but could not be passaged.

Next, the authors generated ALI pancreatic organoids from mice bearing the *Kras*$^{LSL\text{-}G12D/+}$ or *Trp53*$^{fl/fl}$ alleles [4]. Following infection with Adenoviral-*Cre* to activate oncogenic Kras or delete p53, the ALI organoids were able to proliferate and could be serially passaged [4]. *Cre*-infected *Kras*$^{LSL\text{-}G12D/+}$ organoids, *Trp53*$^{fl/fl}$ organoids, or *Kras*$^{LSL\text{-}G12D/+}$; *Trp53*$^{fl/fl}$ double-mutant organoids were all capable of forming tumors when transplanted subcutaneously. While it remains unclear whether PDA tumor cells, which often face hypoxia *in vivo*, will propagate under ALI conditions. However, the ability to model the effects of *Kras* mutation alone or in combination with *Trp53* mutation offers a promising method for studying the steps in tumorigenesis. In addition, the presence of stromal cells in these cultures may better mimic tumor-stroma interactions than models where epithelial cells are cultured alone.

Using Organoids for Personalized Medicine

The relatively rapid generation and high proliferation of organoid cultures makes them well suited for therapeutic research. However, as of this review, there have only been limited examples of therapeutic interrogation using pancreas organoids [6, 7, 98]. Currently, the most comprehensive work in this domain utilizes the intestinal and colonic organoids, and could serve as a model for future pancreas organoid research. Marc van de Wetering and colleagues assembled an organoid biobank from 20 colorectal cancer patients [100]. They performed deep genomic and transcriptomic analyses using both neoplastic and adjacent-normal organoids, thus providing meaningful comparison and identification of tumor-specific DNA and RNA variations. The tumor organoids were screened in a high-throughput manner using a custom library of therapeutic compounds to identify compounds the organoids were sensitive to. This approach led to the identification of effective patient-specific treatments. There was a correlation between therapeutic response and mutational status, confirming previously known, mutation-based drug sensitivities. Importantly, some therapeutic responses could not have been predicted through sequencing analysis alone, highlighting the value of such an approach. To further determine the predictive value of personalized organoid screening, a observational colorectal cancer trial is now underway at the Netherlands Cancer Institute in Amsterdam [101].

An organoid-based personalized medicine approach is also being investigated for the diagnosis and treatment of cystic fibrosis (CF). This clinical advance stems from the work of Johanna Dekkers and colleagues who cultured primary intestinal organoids from CF patients [102]. Due to an inactivating mutation in the *Cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene, the organoids demonstrate poor transmembrane conductance in culture in response to forskolin-induced high intracellular cyclic AMP content, thereby recapitulating a hallmark of cystic fibrosis in a dish. Gerald Schwank and colleagues were able to apply CRISPR/Cas9 genome editing tools to repair the mutant *CFTR* locus in organoid cultures, thus rescuing the CF phenotype [103]. In addition, this defect could be rescued through drug treatment in these *ex vivo* culture models. Distinct *CFTR* mutant organoids responded differently to treatments, and surprisingly, even organoids with the

same *CFTR* mutation displayed differential treatment responses suggesting that organoids may be used to quickly screen and identify the best patient-specific-treatment [102]. If translated to the clinic, such a personalized approach may help CF patients quickly identify therapies they are most likely to benefit from.

The ability to rapidly and efficiently genetically engineer organoids using the CRISPR/Cas9 system also enables cancer research. Two laboratories independently generated isogenic human intestinal organoids lines harboring both tumor suppressor and oncogenic mutations [104, 105]. Using these genetically engineered human organoids, researchers were able to study tumor development and invasive potential. The ability to engineer sequential mutations in human pancreas organoids could aid in the generation of new models based on the newly identified subtypes of the PDA [106].

Bolstered by the results of these preliminary studies, we expect pancreas organoids to advance the state of personalized medicine for PDA. Organoids offer an ideal setting for patient-specific assays as genetic traits of disease such as driver mutations and chromosomal copy number are preserved [10, 107]. The diagnosis of pancreatic cancer often involves collection of a biopsy, such as an endoscopic ultrasound-guided FNA. Unlike many other culture systems, organoids may be cultured from just one needle pass with a high success rate [5]. This enables the possibility of deep genomic characterization and *ex vivo* therapeutic testing in PDA patients that have traditionally been under-studied in research settings. It will be important to determine if these biopsy-derived organoid cultures faithfully represent the genetic heterogeneity and therapeutic sensitivity profile of the entire primary tumor. Conversely, in patients with resectable disease, multiple distinct organoid lines could be generated from cross-sections of the same tumor, and used to model the intratumoral clonal heterogeneity found in PDA patients [108]. Once established, PDA organoid cultures could be expanded, genotyped, and challenged with approved, standard of care therapies within weeks, possibly allowing for organoid-guided, second-line therapeutic options being offered to the patient. A similar approach tested in human liver organoids yielded promising results indicating that the organoid system was amenable to therapeutic testing [109]. Methods are being developed for drug discovery using high throughput testing in 3D cultures which could be applied to therapeutic testing in patient-derived organoids [110]. Finally, a retrospective study using prostate organoids found that the organoids derived from distinct patients displayed different responses to therapies which correlated with the observed genetics of each patient's cancer [10], suggesting that therapeutic testing of organoids will have clinical benefit.

A critical first step for the use of organoids for personalized approaches to PDA care will be an efficacy evaluation within a retrospective clinical trial. In one possible clinical trial, patients with advanced pancreatic cancer would consent to donate a portion of biopsy tissue for the purposes of establishing organoids. Patient-derived organoids would then be used to test therapeutic sensitivities against standard of care drugs, and the results of these assays would be retrospectively compared with the patient's tumor response to therapy. A good correlation between tumor response/progression and organoid sensitivity/resistance to a particular therapeutic regimen would indicate that the organoid system is predictive of treatment outcome. If such a study were successful for PDA, then a prospective study could

be undertaken with the goal of identifying optimal treatments to directly improve patient survival and quality of life. Such studies could also be used to identify second-line treatments following the development of resistance to first-line therapies.

There are numerous challenges to implementing these studies in the clinic. Considering the short median survival of pancreatic cancer patients, the main hurdle to this approach will be to reduce the time necessary to culture and assess the organoids as to offer insight to the clinical care team as soon as possible. Currently, heavy pre-treatment with chemotherapy prior to surgery/biopsy, and low neoplastic cellularity of biopsy tissue are the major factors for failed organoid cultures, which may pose a problem for studying such patients. Optimization of culture methods to more quickly and efficiently generate organoids from such samples may enable sampling of a broader spectrum of patients. A second challenge is the lack of immune, stromal, and vasculature compartments in the organoid culture, thus preventing the effective testing of immuno-therapy (e.g. immune checkpoint inhibitors) or stroma-modulating drugs (e.g. Vitamin D analogues). It will be important for future studies to develop and optimize co-culturing methods to generate a more complete and representative *ex vivo* system.

Before clinical studies using organoids are undertaken, efforts should be made to better characterize PDA organoids. The different organoid methodologies should be compared directly, and protocols for isolation and culturing of PDA organoids should be standardized. Ideally, components involved in the culture matrix and medium would also be standardized. Most current approaches use Matrigel, which has large variation between lots. Synthetic matrices consisting of fully defined components may help to circumvent this issue. These optimization efforts are very important, as human organoid culture is currently an expensive and technically challenging method requiring precious patient samples. Finally, organoid models should be compared to cell line and xenograft approaches to determine the optimal uses for each model.

Concluding Remarks

While there are many questions about organoid cultures that still need to be addressed (see Outstanding Questions), these 3D culture methods have emerged as powerful systems for probing PDA biology. Such methods offer the ability to generate a self-renewing source of patient-specific cells that is amenable to genetic and chemical perturbations. This will allow for basic discoveries into the pathways driving different PDA tumors and for the identification of therapies for PDA. Moreover, organoids are likely to be increasingly incorporated in clinical approaches to PDA care, where they may provide great benefit in guiding individual treatment decisions.

OUTSTANDING QUESTIONS BOX

- Can all neoplastic cells in a tumor propagate as organoids, or do organoid cultures select for certain subclones of the tumor, such as the cancer stem cell population?
- Do neoplastic cells become “reprogrammed” when cultured as organoids?

- Are there other, less expensive culture matrices available that could be used to successfully generate PDA organoids?
- How do the different organoid models compare, and which systems best model human PDA?
- Can mesenchymal cells be added to organoid cultures to better mimic organ architecture and to explore tumor-stroma signaling?
- Do organoids predict which genes are required for human PDA and how human tumors will respond to therapies better than conventional, monolayer cell lines?
- Can the generation of patient-derived organoids be standardized and streamlined enough for organoids to be useful for clinical management of pancreatic cancer patients?

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: a cancer journal for clinicians. 2015; 65(1):5–29. [PubMed: 25559415]
2. Rahib L, et al. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer research. 2014; 74(11):2913–21. [PubMed: 24840647]
3. Ryan DP, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. The New England journal of medicine. 2014; 371(11):1039–49. [PubMed: 25207767]
4. Li X, et al. Oncogenic transformation of diverse gastrointestinal tissues in primary organoid culture. Nature medicine. 2014; 20(7):769–77.
5. Boj SF, et al. Organoid models of human and mouse ductal pancreatic cancer. Cell. 2015; 160(1–2): 324–38. [PubMed: 25557080]
6. Huang L, et al. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. Nature medicine. 2015; 21(11):1364–71.
7. Walsh AJ, et al. Optical Imaging of Drug-Induced Metabolism Changes in Murine and Human Pancreatic Cancer Organoids Reveals Heterogeneous Drug Response. Pancreas. 2015
8. Gey GO, Coffman WD, Kubicek MT. Tissue Culture Studies of the Proliferative Capacity of Cervical Carcinoma and Normal Epithelium. Cancer research. 1952; 12(4):264–265.
9. Dobrynin YV. Establishment and Characteristics of Cell Strains from Some Epithelial Tumors of Human Origin. Journal of the National Cancer Institute. 1963; 31(5):1173–&. [PubMed: 14071827]
10. Gao D, et al. Organoid cultures derived from patients with advanced prostate cancer. Cell. 2014; 159(1):176–87. [PubMed: 25201530]

11. Serebriiskii I, et al. Fibroblast-derived 3D matrix differentially regulates the growth and drug-responsiveness of human cancer cells. Matrix biology : journal of the International Society for Matrix Biology. 2008; 27(6):573–85. [PubMed: 18411046]
12. Birgersdotter A, Sandberg R, Ernberg I. Gene expression perturbation in vitro--a growing case for three-dimensional (3D) culture systems. Seminars in cancer biology. 2005; 15(5):405–12. [PubMed: 16055341]
13. Edmondson R, et al. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay and drug development technologies. 2014; 12(4):207–18. [PubMed: 24831787]
14. Lee J, et al. Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer cell. 2006; 9(5):391–403. [PubMed: 16697959]
15. van Staveren WC, et al. Human cancer cell lines: Experimental models for cancer cells in situ? For cancer stem cells? Biochimica et biophysica acta. 2009; 1795(2):92–103. [PubMed: 19167460]
16. Ertel A, et al. Pathway-specific differences between tumor cell lines and normal and tumor tissue cells. Molecular cancer. 2006; 5(1):55. [PubMed: 17081305]
17. Sandberg R, Ernberg I. Assessment of tumor characteristic gene expression in cell lines using a tissue similarity index (TSI). Proceedings of the National Academy of Sciences of the United States of America. 2005; 102(6):2052–7. [PubMed: 15671165]
18. Stein WD, et al. A Serial Analysis of Gene Expression (SAGE) database analysis of chemosensitivity: comparing solid tumors with cell lines and comparing solid tumors from different tissue origins. Cancer research. 2004; 64(8):2805–16. [PubMed: 15087397]
19. Gadaleta E, et al. A global insight into a cancer transcriptional space using pancreatic data: importance, findings and flaws. Nucleic acids research. 2011; 39(18):7900–7. [PubMed: 21724610]
20. Kim J, et al. An iPSC line from human pancreatic ductal adenocarcinoma undergoes early to invasive stages of pancreatic cancer progression. Cell reports. 2013; 3(6):2088–99. [PubMed: 23791528]
21. Liu X, et al. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. The American journal of pathology. 2012; 180(2):599–607. [PubMed: 22189618]
22. Riento K, Ridley AJ. Rocks: multifunctional kinases in cell behaviour. Nature reviews. Molecular cell biology. 2003; 4(6):446–56. [PubMed: 12778124]
23. Zhang L, et al. ROCK inhibitor Y-27632 suppresses dissociation-induced apoptosis of murine prostate stem/progenitor cells and increases their cloning efficiency. PloS one. 2011; 6(3):e18271. [PubMed: 21464902]
24. Perez-Mancera PA, et al. What we have learned about pancreatic cancer from mouse models. Gastroenterology. 2012; 142(5):1079–92. [PubMed: 22406637]
25. Olive KP, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science. 2009; 324(5933):1457–61. [PubMed: 19460966]
26. Feig C, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110(50):20212–7. [PubMed: 24277834]
27. Ijichi H, et al. Inhibiting Cxcr2 disrupts tumor-stromal interactions and improves survival in a mouse model of pancreatic ductal adenocarcinoma. The Journal of clinical investigation. 2011; 121(10):4106–17. [PubMed: 21926469]
28. Neesse A, et al. CTGF antagonism with mAb FG-3019 enhances chemotherapy response without increasing drug delivery in murine ductal pancreas cancer. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110(30):12325–30. [PubMed: 23836645]
29. Ozdemir BC, et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer cell. 2014; 25(6):719–34. [PubMed: 24856586]
30. Rhim AD, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer cell. 2014; 25(6):735–47. [PubMed: 24856585]

31. Frese KK, et al. nab-Paclitaxel potentiates gemcitabine activity by reducing cytidine deaminase levels in a mouse model of pancreatic cancer. Cancer discovery. 2012; 2(3):260–9. [PubMed: 22585996]
32. Uhl EW, Warner NJ. Mouse Models as Predictors of Human Responses: Evolutionary Medicine. Current pathobiology reports. 2015; 3(3):219–223. [PubMed: 26246962]
33. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. Journal of immunology. 2004; 172(5):2731–8.
34. Calado RT, Dumitriu B. Telomere dynamics in mice and humans. Seminars in hematology. 2013; 50(2):165–74. [PubMed: 23956466]
35. Tomasetti C, Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science. 2015; 347(6217):78–81. [PubMed: 25554788]
36. Biankin AV, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature. 2012; 491(7424):399–405. [PubMed: 23103869]
37. Fogh J, Fogh JM, Orfeo T. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. Journal of the National Cancer Institute. 1977; 59(1):221–6. [PubMed: 327080]
38. Kondo J, et al. Retaining cell-cell contact enables preparation and culture of spheroids composed of pure primary cancer cells from colorectal cancer. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(15):6235–40. [PubMed: 21444794]
39. Johnson JI, et al. Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. British journal of cancer. 2001; 84(10):1424–31. [PubMed: 11355958]
40. Voskoglou-Nomikos T, Pater JL, Seymour L. Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. Clinical cancer research : an official journal of the American Association for Cancer Research. 2003; 9(11):4227–39. [PubMed: 14519650]
41. Hwang CI, et al. Preclinical models of pancreatic ductal adenocarcinoma. The Journal of pathology. 2016; 238(2):197–204. [PubMed: 26419819]
42. Hidalgo M, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer discovery. 2014; 4(9):998–1013. [PubMed: 25185190]
43. Delitto D, et al. Patient-derived xenograft models for pancreatic adenocarcinoma demonstrate retention of tumor morphology through incorporation of murine stromal elements. The American journal of pathology. 2015; 185(5):1297–303. [PubMed: 25770474]
44. Gao H, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nature medicine. 2015; 21(11):1318–25.
45. Rubio-Viqueira B, et al. An in vivo platform for translational drug development in pancreatic cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2006; 12(15):4652–61. [PubMed: 16899615]
46. Fu X, Guadagni F, Hoffman RM. A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically with histologically intact patient specimens. Proceedings of the National Academy of Sciences of the United States of America. 1992; 89(12):5645–9. [PubMed: 1608975]
47. Loukopoulos P, et al. Orthotopic transplantation models of pancreatic adenocarcinoma derived from cell lines and primary tumors and displaying varying metastatic activity. Pancreas. 2004; 29(3):193–203. [PubMed: 15367885]
48. Garrido-Laguna I, et al. Tumor engraftment in nude mice and enrichment in stroma-related gene pathways predict poor survival and resistance to gemcitabine in patients with pancreatic cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2011; 17(17):5793–800. [PubMed: 21742805]
49. Hidalgo M, et al. A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. Molecular cancer therapeutics. 2011; 10(8):1311–6. [PubMed: 21673092]
50. Aparicio S, Hidalgo M, Kung AL. Examining the utility of patient-derived xenograft mouse models. Nature reviews. Cancer. 2015; 15(5):311–6.
51. Carrel, A.; Lindberg, CA. The Culture of Organs. New York: Paul B. Hoeber, Inc; 1938.

52. Jones RT, Hudson EA, Resau JH. A review of in vitro and in vivo culture techniques for the study of pancreatic carcinogenesis. Cancer. 1981; 47(6 Suppl):1490–6. [PubMed: 6791803]
53. Githens S 3rd, et al. Ducts of the rat pancreas in a agarose matrix culture. In vitro. 1980; 16(9): 797–808. [PubMed: 7409838]
54. Montesano R, et al. Collagen matrix promotes reorganization of pancreatic endocrine cell monolayers into islet-like organoids. The Journal of cell biology. 1983; 97(3):935–9. [PubMed: 6350323]
55. Kerr-Conte J, et al. Ductal cyst formation in collagen-embedded adult human islet preparations. A means to the reproduction of nesidioblastosis in vitro. Diabetes. 1996; 45(8):1108–14. [PubMed: 8690159]
56. Marciniak A, et al. Mouse pancreas tissue slice culture facilitates long-term studies of exocrine and endocrine cell physiology in situ. PloS one. 2013; 8(11):e78706. [PubMed: 24223842]
57. van Geer MA, et al. Ex-vivo evaluation of gene therapy vectors in human pancreatic (cancer) tissue slices. World journal of gastroenterology. 2009; 15(11):1359–66. [PubMed: 19294766]
58. Vaira V, et al. Preclinical model of organotypic culture for pharmacodynamic profiling of human tumors. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107(18):8352–6. [PubMed: 20404174]
59. Alagesan B, et al. Combined MEK and PI3K inhibition in a mouse model of pancreatic cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2015; 21(2):396–404. [PubMed: 25348516]
60. Schreiber FS, et al. Successful growth and characterization of mouse pancreatic ductal cells: functional properties of the Ki-RAS(G12V) oncogene. Gastroenterology. 2004; 127(1):250–60. [PubMed: 15236190]
61. Agbunag C, et al. Pancreatic duct epithelial cell isolation and cultivation in two-dimensional and three-dimensional culture systems. Methods in enzymology. 2006; 407:703–10. [PubMed: 16757363]
62. Lee KE, Bar-Sagi D. Oncogenic KRas suppresses inflammation-associated senescence of pancreatic ductal cells. Cancer cell. 2010; 18(5):448–58. [PubMed: 21075310]
63. Botta GP, et al. Constitutive K-RasG12D activation of ERK2 specifically regulates 3D invasion of human pancreatic cancer cells via MMP-1. Molecular cancer research : MCR. 2012; 10(2):183–96. [PubMed: 22160930]
64. Sempere LF, Gunn JR, Korc M. A novel 3-dimensional culture system uncovers growth stimulatory actions by TGFbeta in pancreatic cancer cells. Cancer biology & therapy. 2011; 12(3): 198–207. [PubMed: 21613822]
65. Fanjul M, Hollande E. Morphogenesis of "duct-like" structures in three-dimensional cultures of human cancerous pancreatic duct cells (Capan-1). In vitro cellular & developmental biology. Animal. 1993; 29A(7):574–84. [PubMed: 8354666]
66. Matsuda Y, et al. Morphological and cytoskeletal changes of pancreatic cancer cells in three-dimensional spheroidal culture. Medical molecular morphology. 2010; 43(4):211–7. [PubMed: 21267697]
67. Kim MP, et al. ALDH activity selectively defines an enhanced tumor-initiating cell population relative to CD133 expression in human pancreatic adenocarcinoma. PloS one. 2011; 6(6):e20636. [PubMed: 21695188]
68. Wen Z, et al. A spheroid-based 3-D culture model for pancreatic cancer drug testing, using the acid phosphatase assay. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas /Sociedade Brasileira de Biofisica ... [et al.]. 2013; 46(7):634–42.
69. Yeon SE, et al. Application of concave microwells to pancreatic tumor spheroids enabling anticancer drug evaluation in a clinically relevant drug resistance model. PloS one. 2013; 8(9):e73345. [PubMed: 24039920]
70. McLeod EJ, et al. Multicellular tumor spheroids grown from pancreatic carcinoma cell lines: use as an orthotopic xenograft in athymic nude mice. Pancreas. 1997; 14(3):237–48. [PubMed: 9094153]
71. Gou S, et al. Establishment of clonal colony-forming assay for propagation of pancreatic cancer cells with stem cell properties. Pancreas. 2007; 34(4):429–35. [PubMed: 17446842]

72. Grzesiak JJ, Bouvet M. Determination of the ligand-binding specificities of the alpha2beta1 and alpha1beta1 integrins in a novel 3-dimensional in vitro model of pancreatic cancer. Pancreas. 2007; 34(2):220–8. [PubMed: 17312461]
73. Gutierrez-Barrera AM, et al. Establishment of three-dimensional cultures of human pancreatic duct epithelial cells. Biochemical and biophysical research communications. 2007; 358(3):698–703. [PubMed: 17512909]
74. Hosoya H, et al. Engineering fibrotic tissue in pancreatic cancer: a novel three-dimensional model to investigate nanoparticle delivery. Biochemical and biophysical research communications. 2012; 419(1):32–7. [PubMed: 22321398]
75. Longati P, et al. 3D pancreatic carcinoma spheroids induce a matrix-rich, chemoresistant phenotype offering a better model for drug testing. BMC cancer. 2013; 13:95. [PubMed: 23446043]
76. Ricci C, et al. Interfacing polymeric scaffolds with primary pancreatic ductal adenocarcinoma cells to develop 3D cancer models. Biomatter. 2014; 4:e955386. [PubMed: 25482337]
77. Froeling FE, et al. Organotypic culture model of pancreatic cancer demonstrates that stromal cells modulate E-cadherin, beta-catenin, and Ezrin expression in tumor cells. The American journal of pathology. 2009; 175(2):636–48. [PubMed: 19608876]
78. Kadaba R, et al. Imbalance of desmoplastic stromal cell numbers drives aggressive cancer processes. The Journal of pathology. 2013; 230(1):107–17. [PubMed: 23359139]
79. Wang YJ, et al. Sphere-forming assays for assessment of benign and malignant pancreatic stem cells. Methods in molecular biology. 2013; 980:281–90. [PubMed: 23359160]
80. Rovira M, et al. Isolation and characterization of centroacinar/terminal ductal progenitor cells in adult mouse pancreas. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107(1):75–80. [PubMed: 20018761]
81. Seaberg RM, et al. Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. Nature biotechnology. 2004; 22(9):1115–24.
82. Hermann PC, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell stem cell. 2007; 1(3):313–23. [PubMed: 18371365]
83. Pastrana E, Silva-Vargas V, Doetsch F. Eyes wide open: a critical review of sphere-formation as an assay for stem cells. Cell stem cell. 2011; 8(5):486–98. [PubMed: 21549325]
84. Katano T, et al. Establishment of a long-term three-dimensional primary culture of mouse glandular stomach epithelial cells within the stem cell niche. Biochemical and biophysical research communications. 2013; 432(4):558–63. [PubMed: 23485463]
85. Bartfeld S, et al. In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. Gastroenterology. 2015; 148(1):126–136 e6. [PubMed: 25307862]
86. Barker N, et al. Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell stem cell. 2010; 6(1):25–36. [PubMed: 20085740]
87. Ootani A, et al. Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. Nature medicine. 2009; 15(6):701–6.
88. Sato T, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009; 459(7244):262–5. [PubMed: 19329995]
89. Sato T, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 2011; 141(5):1762–72. [PubMed: 21889923]
90. Huch M, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell. 2015; 160(1–2):299–312. [PubMed: 25533785]
91. Huch M, et al. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. Nature. 2013; 494(7436):247–50. [PubMed: 23354049]
92. Petersen OW, et al. Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. Proceedings of the National Academy of Sciences of the United States of America. 1992; 89(19):9064–8. [PubMed: 1384042]

93. Schwarz JS, de Jonge HR, Forrest JN Jr. Value of Organoids from Comparative Epithelia Models. The Yale journal of biology and medicine. 2015; 88(4):367–74. [PubMed: 26604860]

94. Greggio C, et al. Artificial three-dimensional niches deconstruct pancreas development in vitro. Development. 2013; 140(21):4452–62. [PubMed: 24130330]

95. Huch M, et al. Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. The EMBO journal. 2013; 32(20):2708–21. [PubMed: 24045232]

96. Huch M, Koo BK. Modeling mouse and human development using organoid cultures. Development. 2015; 142(18):3113–25. [PubMed: 26395140]

97. Dorrell C, et al. The organoid-initiating cells in mouse pancreas and liver are phenotypically and functionally similar. Stem cell research. 2014; 13(2):275–83. [PubMed: 25151611]

98. Kumar K, et al. Differential Regulation of ZEB1 and EMT by MAPK-interacting protein kinases (MNKs) and eIF4E in Pancreatic Cancer. Molecular cancer research : MCR. 2015

99. Stoker MG, Shearer M, O'Neill C. Growth inhibition of polyoma-transformed cells by contact with static normal fibroblasts. Journal of cell science. 1966; 1(3):297–310. [PubMed: 4291022]

100. van de Wetering M, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell. 2015; 161(4):933–45. [PubMed: 25957691]

101. Clevers H, Bender E. Q&A: Hans Clevers. Banking on organoids. Nature. 2015; 521(7551):S15. [PubMed: 25970453]

102. Dekkers JF, et al. A functional CFTR assay using primary cystic fibrosis intestinal organoids. Nature medicine. 2013; 19(7):939–45.

103. Schwank G, et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell stem cell. 2013; 13(6):653–8. [PubMed: 24315439]

104. Matano M, et al. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. Nat Med. 2015; 21(3):256–62. [PubMed: 25706875]

105. Drost J, et al. Sequential cancer mutations in cultured human intestinal stem cells. Nature. 2015; 521(7550):43–7. [PubMed: 25924068]

106. Bailey P, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature. 2016

107. Weeber F, et al. Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112(43):13308–11. [PubMed: 26460009]

108. Yachida S, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature. 2010; 467(7319):1114–7. [PubMed: 20981102]

109. Skardal A, et al. Liver-Tumor Hybrid Organoids for Modeling Tumor Growth and Drug Response In Vitro. Ann Biomed Eng. 2015; 43(10):2361–73. [PubMed: 25777294]

110. Eglen RM, Randle DH. Drug Discovery Goes Three-Dimensional: Goodbye to Flat High-Throughput Screening? Assay Drug Dev Technol. 2015; 13(5):262–5. [PubMed: 26121065]

111. Blow N. Cell culture: building a better matrix. Nature methods. 2009; 6(8):619–622.

112. Gurski LA, et al. 3D matrices for anti-cancer drug testing and development. Oncology issues. 2010 Jan-Feb;:20–25.

113. Gelse K, Poschl E, Aigner T. Collagens--structure, function, and biosynthesis. Advanced drug delivery reviews. 2003; 55(12):1531–46. [PubMed: 14623400]

114. Amsterdam A, Jamieson JD. Studies on dispersed pancreatic exocrine cells. I. Dissociation technique and morphologic characteristics of separated cells. The Journal of cell biology. 1974; 63(3):1037–56. [PubMed: 4373477]

115. Cirulli V, et al. Expression and function of alpha(v)beta(3) and alpha(v)beta(5) integrins in the developing pancreas: roles in the adhesion and migration of putative endocrine progenitor cells. The Journal of cell biology. 2000; 150(6):1445–60. [PubMed: 10995448]

116. Shields MA, et al. Biochemical role of the collagen-rich tumour microenvironment in pancreatic cancer progression. The Biochemical journal. 2012; 441(2):541–52. [PubMed: 22187935]

117. Ohlund D, et al. Type IV collagen is a tumour stroma-derived biomarker for pancreas cancer. British journal of cancer. 2009; 101(1):91–7. [PubMed: 19491897]

118. Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. Seminars in cancer biology. 2005; 15(5):378–86. [PubMed: 15975825]

119. Vukicevic S, et al. Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components. Experimental cell research. 1992; 202(1):1–8. [PubMed: 1511725]

120. Taub M, et al. Epidermal growth factor or transforming growth factor alpha is required for kidney tubulogenesis in matrigel cultures in serum-free medium. Proceedings of the National Academy of Sciences of the United States of America. 1990; 87(10):4002–6. [PubMed: 2339133]

TRENDS BOX

- Pancreatic ductal adenocarcinoma (PDA) is an extremely aggressive and lethal malignancy. There is an urgent need for research advances that could help lead to improved outcomes and earlier diagnoses for PDA.
- While the mainstays of PDA research have traditionally been genetically engineered mouse models, PDA cell lines cultured as monolayers, and patient-derived xenografts, each of these model systems suffers from limitations.
- Organotypic culture methods, in which cells are cultured in three-dimensions under conditions meant to mimic those in the body, have emerged as powerful new systems to circumvent some of the limitations of other models.
- Recently, four different methods for modeling PDA as organotypic, organoid cultures have been reported. These methods allow researchers to rapidly make new discoveries about pancreatic tumorigenesis that are highly relevant to human patients.

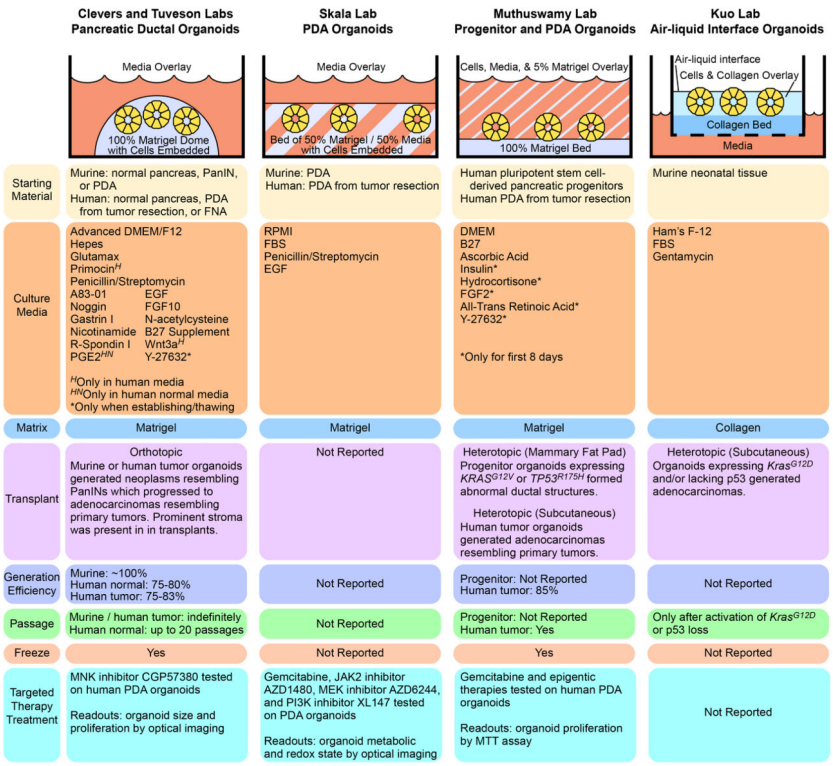

Figure 1. Organoid models of pancreatic ductal adenocarcinoma

Diagrams of a cross-section of a single organoid culture well for each of the four recently reported methods for modeling PDA using organoids, the Clevers and Tuveson Laboratories method [5], the Skala Laboratory method [7], the Muthuswamy Laboratory method [6], and the Kuo Laboratory method [4]. Characteristics of each system are shown below diagrams. Matrix components are depicted in blue, culture medium is in orange, and organoids are in yellow. The targeted therapeutic study using organoids cultured with the Clevers and Tuveson organoid method is described in Ref [98]. MNK: mitogen activated protein kinase-interacting protein kinase. JAK2: Janus kinase 2. MEK: mitogen-activated protein kinase kinase. PI3K: phosphoinositide 3-kinase. FNA: Fine needle aspirate.