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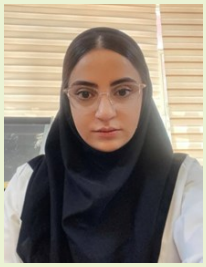
In Vivo and In Vitro Models of Pancreatic Cancer: A Comparative Study

Nazanin Jamshidi[1] , Aniya Ghasemzadeh[2] , Negar jamshidi[1] , Vahid Chaleshi[1]*

[1]Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Biology, Parand Branch, Islamic Azad University, Parand, Iran
***Corresponding Author: Vahid Chaleshi Tel: +98-2122432525, Fax +98-2122432514, E-mail: chaleshi@gmail.com, chaleshi@sbmu.ac.ir**



Nazanin Jamshidi
Ph.D. Student of Cellular and Molecular Biology

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Abstract

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer and has an extremely low 5-year survival rate of less than 10%, making it one of the deadliest solid tumors. To improve our understanding of the genesis and progression of this devastating disease, novel laboratory approaches have been developed. In this review, we provide a comprehensive summary of the current in vitro and in vivo models of PDAC, including established murine and human PDAC cell lines, organoids, and genetically engineered models. In addition, we compare these models and analyze published in vitro and in vivo PDAC models to elucidate the molecular basis of pancreatic carcinogenesis.

Keywords: Pancreatic ductal adenocarcinoma, in vitro models ,in vivo models

1. Introduction

Pancreatic cancer (PC) is a highly aggressive form of cancer and the fourth leading cause of cancer-related deaths in the United States(1). Due to its asymptomatic nature, until it has metastasized, it is difficult to diagnose and treat. PDAC is the most common type of PC, originating from the cells that line the ducts of the pancreas, while neuroendocrine tumors and sarcomas are less common. The standard treatment for pancreatic cancer includes surgery(2), chemotherapy (3), radiation(4) therapy, or a combination of these therapies (1).

Despite significant advances in systemic therapies, the prognosis for patients with PDAC remains poor, with a median survival time of less than a year (5). Due to the complex interplay of mutational activity, intra- and intercellular signaling pathways, and cellular features of the tumor, personalized therapeutic strategies are needed for PDAC patients. Therefore, adequate preclinical models are essential to study the disease and develop effective treatments (6).

animals, like mice, are used in in vivo models to learn more about the illness. It is possible to research the impact of medications and therapies on cancer progression and evaluate the efficacy of proposed treatments by using these models, which more closely mimic the human disease process (6).In contrast, in vitro models are conducted in a laboratory setting, using cell cultures and other artificial systems.

Although limited in their ability to mimic the disease process, they are useful for studying the underlying biology of the disease, testing the effects of potential treatments, and identifying biomarkers for early detection and diagnosis of pancreatic cancer (6, 7).An in vitro model of pancreatic cancer can be created by culturing pancreatic cancer cells in a laboratory(6). This can be done by isolating cancer cells from a patient's sample, such as a biopsy, and culturing them in a cell culture dish. This allows researchers to study the cancer cells and their behavior in a controlled environment. Additionally, this model can be used to test the effects of new drugs and treatments on pancreatic cancer cells(8).

One type of in vitro model for pancreatic cancer treatment is a three-dimensional (3D) cell culture system(9). This model uses a combination of pancreatic cancer cells, fibroblasts, and extracellular matrix proteins to create a 3D structure that mimics the in vivo environment of a pancreatic tumor. This model allows researchers to study the effects of various treatments, such as chemotherapies and targeted therapies, on the tumor cells in a more realistic environment(10).

The organoid model of pancreatic cancer is a 3D culture system that is used to study the development and progression of pancreatic cancer(11). This model is derived from patient-derived cells, which are grown in a culture medium that mimics the in vivo environment. This allows for the study of the interactions between cancer cells and the surrounding microenvironment, as well as the effects of potential treatments. The organoid model can be used to study the genetic and epigenetic alterations that occur in pancreatic cancer, as well as the effects of drugs on the tumor. Additionally, this model can be used to study the effects of radiation and chemotherapy, as well as the development of resistance to these treatments(12).

In this study, we aimed to compare the advantages and limitations of in vivo and in vitro models of pancreatic cancer. It is important to note that both models have their own unique advantages and disadvantages and should be used in conjunction with one another to provide a more comprehensive understanding of the disease.

2. In vivo modeling of pancreatic cancer

In vivo modeling of pancreatic cancer can be done using mouse models that have been genetically engineered to develop pancreatic tumors. These models are useful for studying the biology of pancreatic cancer, for testing potential new treatments, and for understanding how the disease progresses over time(13, 14).

Currently, there are several in vivo models available for PDAC, including xenografts, patient-derived xenografts (PDX), a wide range of genetic mouse models, and syngeneic xenografts. Each model has its own set of advantages and disadvantages, and here we summarize the in vivo diverse models and some of the issues related to their application.

2.1. Xenograft Mouse model

A xenograft is the transfer of organs, tissue, or cells from one species to another, in which human cells or tissues are transferred to laboratory mice to create a xenograft (15). Laboratory mice have long been used as models for assessing the development and treatment of human diseases. However, the transfer of human cells to mice is not easily accomplished because human cells are rapidly recognized and destroyed by the mouse immune system. To overcome this problem, diverse mouse models of immunodeficiency have been produced (16). The nude mouse was the first mouse model for studying human cells (17). These mice lack T-cells and can receive human cancer cells, leading to tumor growth in these models due to a mutation in the forkhead box gene N1 (FOXN1). Nevertheless, nude mice have active B lymphocytes and a significant number of natural killer (NK) cells (18). After the discovery of athymic nude mice, severe combined immunodeficient mice (SCID) were also presented, which had a spontaneous recessive autosomal mutation in the Protein Kinase, DNA-Activated, Catalytic Subunit (Prkdcscid) gene that severely disturbs the lymphaticsystem and results in deficient B- and T-cells (19).

However, the xenograft model has some limitations due to the high level of heterogeneity in cancer, which mostly results from genetic instability (20). Moreover, characteristics of cancer cells that are isolated from different stages of the same patients (primary tumors or metastases) and even distinct regions of primary tumors may vary (21, 22).

On the other hand, cancer cell populations can adapt to condition changes due to their inherent genetic instability. After in vitro passage, many cell lines no longer represent the native tumor (23).

Despite the high costs of the xenograft model, low power for screening the possible treatments, and the impact of personal cancer genotype on treatment, this model has become a popular one. The advantages of the xenograft models are that they are easy to generate tumor fragments, have consistent tumor growth, have similar histology and genomics with the initial tumor, and have a predictable model. However, the immune system of mice and the creation of mouse stroma do not resemble those of humans, and the model cannot show the complex human tumor heterogeneity and tumor microenvironment (24). Xenografts can be classified into two groups based on the production method: cell-derived xenografts (CDX) and patient-derived xenografts (PDX)(Figure 1)

CDX is a model that uses a cell line as a primary source of tumor production by injecting cells into immunodeficient mice, and the tumor forms within 4 to 6 weeks (25) (Figure1A). Although this model is easy to produce, tumor growth can continue for several generations in mice, and it does not adequately reflect the patient's drug response (26). In addition, CDX tumor cells are surrounded by mouse fibroblasts, vascular cells, and immune cells, and interaction with these cells is a vital factor in the formation of the tumor microenvironment and characteristics of any tumor.

PDX is a more advanced model resulting from the tumor specimen being transplanted directly into an immunodeficient mouse (Figure 1B). PDX xenografts can transfer to new mice with high tumor growth rates when they reach a suitable logarithmic phase (27, 28). Unlike the CDX model, there is no intermediate laboratory processing step before the implanted tumor components in the mouse (29). Instead, for easier transplantation, the tumor pieces should be shredded to 2-3 mm. Immunodeficient mice are used to prevent transplant rejection, and

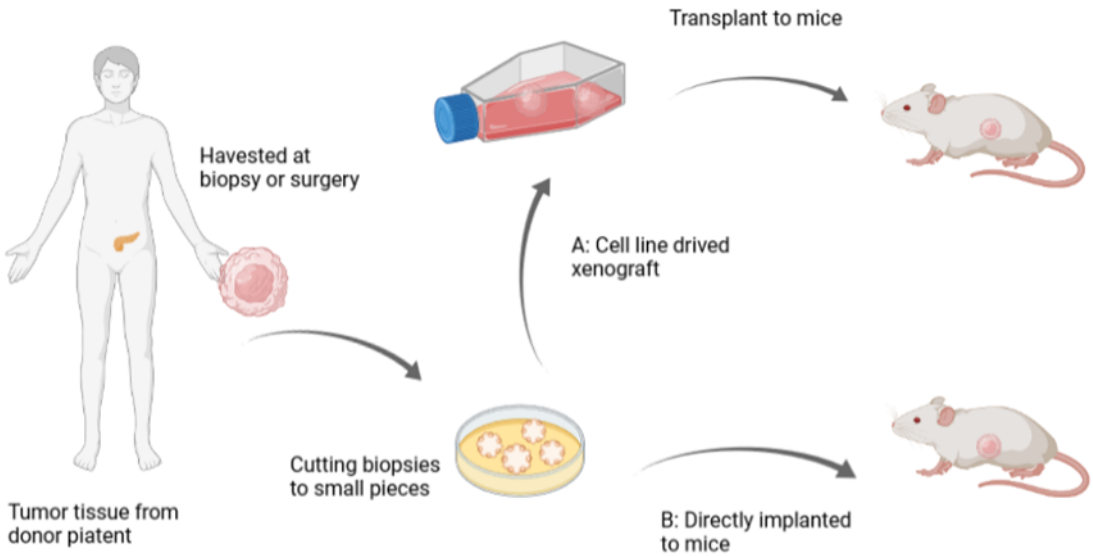

Figure 1.Shematic view of CDX(A) and PDX(B).

different mice such as athymic nude, severely compromised immune deficient (SCID), nonobese diabetic (NOD)- SCID mice, and recombination-activating gene 2 (Rag2)- knockout mice are used to create this model (30). PDX models have been successfully developed for cancers of the breast, prostate, pancreas, colon, lung, and many others to evaluate the safety and efficacy of the drug, as well as to examine the individual response to certain anticancer agents (31). This model has several benefits, such as allowing tumors to maintain cell-cell interactions and form a microenvironment. Therefore, tissue characteristics and tumor histology are preserved in a xenograft model (32). In addition, this model well presents the genomic characteristics of primary tumors. Another benefit of this model is maintaining human stroma in the initial stages of passage. However, the tumor-related stroma is unfortunately replaced by murine stromal cells, such as blood vessels and fibroblasts (33). Although PDX can be partially stabilized by murine stroma, the difference in the ligand-receptor complex can affect tumor phenotype (34). Moreover, PDX tumors may result from a low amount of patient tumors. PDX is a useful resource model for cancer studies because it can be applied to mice for several generations (35).

On the other hand, this model has some limitations. First, it is time-consuming; second, human tumor stromal cells and extracellular matrix (ECM) implanted into immunocompromised mice can be replaced by mouse tissue, and so do not entirely mimic human microenvironments. Third, if the donor patient has an infection of the Epstein-Barr virus, PDX transplantation can cause lymphoma and not cause the expected tumors in the host mouse. Fourth, to produce the suitable xenografts, the immune systems of the mice must be suppressed, and as a result, the xenograft mouse model may not be appropriate for immune response research (36).

Some investigations managed to produce PDX for pancreatic cancer. Mike Mattie et al. in 2013, developed a PDX panel of esophageal and pancreatic malignancies, and they examined the molecular characteristics of eight pancreatic ductal adenocarcinoma patient tumors and compared them with pieces tumors obtained by xenografts grafting mice. These PDXs were representative of the histologic, biochemical, and immunologic characteristics of the main tumors (37).

Mattie et al.'s analysis of mutations revealed that PIK3CA and KRAS mutations were similar to a mutation in PDX (37). Daniel Delitto et al. in 2015, designed and generated PDX for pancreatic adenocarcinoma, and they found that murine stroma cells interacted with human cancer cells; hence, this model cannot fully represent human models (38). Qichen et al. in 2020 designed a PDX model in the first generation of nude mice of tumors isolated from PC to identify the main determinant factors in PDX formation. In addition, they determined the potential of this model as a predictor of disease prognosis. The initial tumor samples were prepared from PC patients under surgery from May 2016-April 2018 and were xenografted into first-generation mice. They compared the pathologic and genetic characteristics of patients with PC tumors in the PDX model of nude mice (39).

As a result, determining which biological characteristics are critical and whether xenograft models are adequate necessitates extensive modeling of various models.

2.2. Genetically Engineered Mouse Models (GEMMs)

The predominant role of genetic factors in the development of most cancers has been suggested by research studies. Therefore, mouse models that mimic genetic heterogeneity in humans can be highly useful and efficient in studying cancer. Genetic engineering has enabled researchers to activate and inactivate a series of genes in mice, and make genetic edits in the mouse genome to design genetically engineered mouse models (GEMMs) for studying disease progression from early stages to metastasis (40, 41).

In GEMMs, the incidence of carcinogenic KRAS mutation is sufficient to achieve histologically confirmed pancreatic ductal adenocarcinoma (PDAC) tumors in mice. The model provides the possibility for tumor growth and metastasis in a correct microenvironment along with intrinsic fibrosis, stroma, and a highly efficient immune system. Additionally, GEMMs allow access to PDAC in the initial stages of the disease. Therefore, GEMMs are ideal for evaluating the impact of specific genetic alterations on the initiation and development of PDAC. However, the generation and storage of these models are very costly and time-consuming .

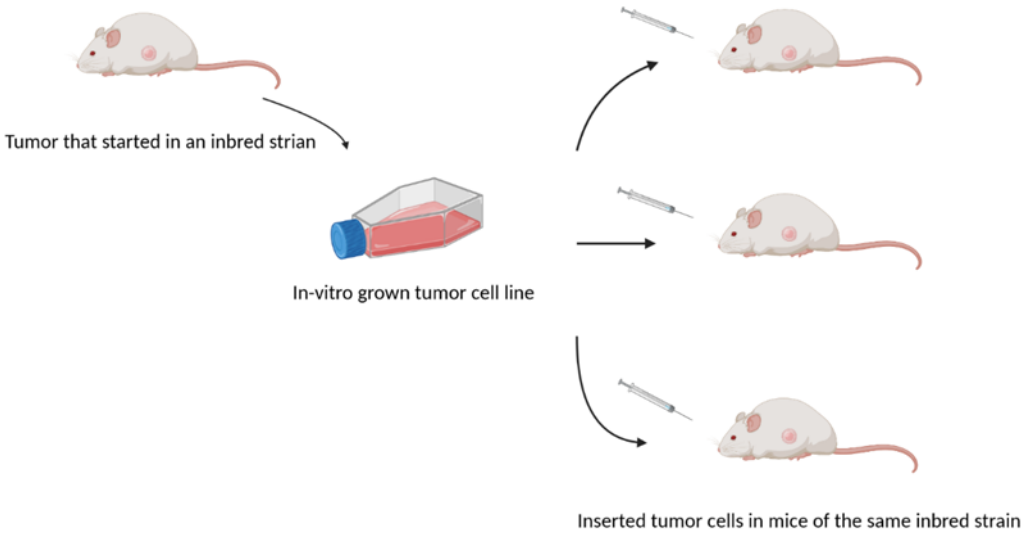

Figure 2- Syngeneic mouse models generation method for immunotherapy studies

Moreover, mutations involve sex cells in addition to somatic cells (42) .

2.3. Immunocompetent Mouse Models: Syngeneic Mouse Models

In order to assess the importance of immunity in cancer, researchers have designed syngeneic mouse models, which involve grafting murine cancer cells onto the same genetic background mouse to overcome the problem of rejection (43). First, cells are derived from the tumor tissue of mice, and then the mouse cancer cells are transferred to the same genetic background mouse (Figure 2). Since syngeneic mouse models maintain an intact immune system, they are important for studying immunotherapy. The Panc02 cell line, derived from the pancreatic tumor in C57BL/6 mice, was developed in 1984 for genetic investigations on pancreatic cancer and evaluation of the immune system's importance (44). However, the genetic characteristics of mouse cell lines do not fully represent the common genetic variations in human pancreatic ductal adenocarcinoma (PDAC), including mutations in K-ras, P53, or P16, but only the deletion of SMAD4 (45). Fortunately, the development of genetically engineered mouse models (GEMMs) for PDAC has led to the development of more mouse cancer cell lines (46).

An ideal model should reflect the structure and genetic characteristics of the tissue under study, including its heterogeneity and stages of disease, as well as its responsiveness to stimuli. Furthermore, this model should be practically repeatable, reproducible, easy to maintain, and inexpensive. Therefore, while in vitro models have numerous strengths and applications, they are not complete and need to be complemented with more efficient and beneficial models for the treatment and prognosis of cancer.

3.In Vitro Modeling of Pancreatic Cancer

Researchers have proposed diverse in vitro models for investigating stem cells, the mechanisms of cancer proliferation and aggression, and medication toxicity. These models include 2D culture, co-culture, and 3D culture(6, 47, 48). Here, we summarize the details of in vitro models and their types in pancreatic cancer cells.

3.1. 2D Cell Culture

2D culture is one of the most commonly used cell culture techniques, in which cells attach to glass and/or plastic beds and grow as a monolayer (49, 50). The 2D culture has allowed for a biological understanding of the mechanisms of diseases, medication action, and protein production (51). This method has been widely used in clinical studies on pharmacological agents and the performance of genes involved in cancer (52).

The benefits of 2D culture include its easy usage, minimum costs, the possibility of genetic and pharmacologic manipulation, rapid and unlimited culture of cells in laboratory environments (43), and the opportunity to evaluate the toxicity of novel combinations (53).

However, these cell lines have some limitations, such as a lack of cell-cell and cell-matrix interactions,

which are involved in cell differentiation, cell proliferation, and viability, changes in gene expression in transcriptome and proteome levels, response to stimuli, medicine metabolism, and other cellular functions (54). Moreover, only one cell type can be cultured in this model, making the investigation of microenvironments impossible (55).

Furthermore, cell morphology (56), cell function, structure, and cellular signaling (57)can alter following the isolation of cells from the primary tissue and transfer to 2D conditions. Additionally, cells lose their polar growth (58), and their cellular sensitivity and responses to environmental stimuli are different from in vivo conditions. In addition, apoptosis is induced in 2D cell lines more rapidly and sensitively (59). The consumption of oxygen, nutrients, and metabolites by cancer cells varies based on tumor structure, whereas cells have unlimited access to nutrients, oxygen, and metabolites in 2D culture (60).

3.2. Co-culture Models

Mostly only one cell type is utilized in cell-based in vitro models, which cannot accurately represent the complexity of in vivo environments with the simultaneous interaction of several cells. As a result, researchers are attempting to design models for optimizing tumor conditions in vitro. One of these models is the co-culture model, in which two or several distinct cell types are cultured together in a plate or well (61).

Co-cultures are categorized into two groups: direct and indirect. In direct co-culture, cells grow as overlaying layers and are in direct contact. Indirect co-culture is defined as the interaction of cells via permeable membranes (62, 63). Direct co-culture is applied for studies on the physical interactions between two cell populations, such as molecule adhesion, cytokines production, and signal production. On the other hand, indirect co-culture is not acceptable for assessing adhesion and cytokines. The latter technique is beneficial for evaluating paracrine signaling in cellular alterations of special populations (64).

Animal models, 2D cell culture, and co-culture models have been widely used in tumor research to assess drug bioavailability, therapeutic efficacy, and dose-limiting toxicity (61). However, these models have some limitations, including high costs, species differences, and limited availability and feasibility (65). Additionally, ethical concerns regarding the use of animals in tumor research are a highly debated issue. The first guiding principle of animal models is to replace animals with alternative methods whenever possible (66). Consequently, funding agencies are encouraging the development of novel in vitro cell culture models to reduce the number of animals used in tumor research and drug evaluation (67, 68).

To address these limitations, 3D tumor cell culture methods have been developed that take into account the spatial organization and extracellular matrix of cells in the culture environment. The ultimate goal of these techniques is to create a biomimetic 3D multicellular tumor model that can bridge the gap between conventional 2D in vitro and animal testing models. Tumor cells grown in 3D models exhibit physiological properties that are more similar to those of in vivo tumors (69, 70). Thus, 3D culture has emerged as a powerful tool in tumor research and drug evaluation. Significant advances have been made in the development of 3D tumor models, and this review provides an overview of the methods and techniques that have been successfully employed for 3D tumor cell culture.

3.3. 3D Cell Culture

In vivo, most cells are surrounded by other cells and extracellular matrix in a 3D manner. Therefore, traditional 2D cell culture models fail to provide natural cell conditions adequately, which has led to the development of 3D culture systems that almost eliminate these problems (66, 67). Several studies have shown that 3D cell culture models reflect cellular responses in the in vivo environment more accurately than 2D culture systems (71). 3D culture systems have many benefits, including the evaluation of cellular function, behavior, morphology, gene expression, paracrine and cell-cell contacts, and the examination of interactions between specific cell types, all at a lower cost than mouse models(Table1) (69, 70).Additionally, 3D culture systems are more similar to solid tumors in terms of proliferation, differentiation, and expression of different genes compared to their 2D counterparts (72). Furthermore, 3D systems mimic metabolic activity, response to cellular stress, structure, signal transduction, and cellular transfer proteins with greater accuracy than 2D systems (73, 74).

Table 1 summarizes the advantages and disadvantages of 2D and 3D cultures

	2D culture	3D culture
Advantages	Easy to use and maintain, lower cost, Reproducible	Better mimicry of in vivo environment, better evaluation of cellular function, behavior, morphology, gene expression, paracrine and cell-cell contacts, examination of interactions between specific cell types, better similarity to solid tumors, better mimicry of metabolic activity, response to cellular stress, structure, signal transduction,
Disadvantages	Poor mimicry of in vivo environment, limited cellular function evaluation	Reproducibility, scalability, Cost

4. The 3D model can be divided into two sections

4.1. 3D oncology studies and drug-resistant

The phenotypic heterogeneity of cancer cells(75), the cellular context, heterotypic crosstalk, and the microenvironment play critical roles in the multistep process of tumor development. These factors are also responsible, to a great extent, for the limited response and resistance of cancer cells to molecular-targeted therapies. Therefore, a better functional understanding of the complex intra- and intercellular signaling circuits underlying communication between the different cell types populating a tumor tissue and of the systemic and local factors that shape the tumor microenvironment is essential(76).

To address these challenges, a range of 3D cell culture techniques have been developed that can be applied to various research applications, including cancer modeling and drug discovery. The advantages of using cells grown in 3D culture conditions over 2D culture models for evaluating drug candidates and exploring the mechanistic properties of anti-cancer agents include: (i) oxygen and nutrient gradients, (ii) increased cell-to-cell interactions resulting from the cellular formation into 3D architecture, (iii) non-uniform exposure of cells within a spheroid to drug/compound, (iv) ECM-to-cell signaling, (v) different rates of cellular proliferation throughout the 3D structure, and (vi) the impact of stromal/tumor site-specific cells in the tumor microenvironment (77).

For example, hepatic cancer cells grown in 3D culture have demonstrated drug resistance characteristics similar to those of solid tumors in vivo (78). Similarly, breast cancer MCF-7 cells in 3D scaffolds showed stronger resistance to tamoxifen in endocrine therapy than those in monolayer culture (79).

4.1.1. organoids for genetic studies

Organoids represent another 3D cell culture model that has gained considerable attention for genetic studies.

Organoids are three-dimensional cell cultures that mimic the structure and function of organs. These cultures have been generated from primary normal tissue as well as primary tumor cells. Organoids offer several advantages for therapeutic research, including their relative rapid generation and high proliferation rate. However, as of this review, there have been only limited examples of therapeutic interrogation using pancreas organoids (80-82).

Organoid models have been described for the stomach (83-85), small intestine, colon (86, 87) liver (88, 89)

mammary gland (90), as well as numerous other tissues (91). Additionally, tumor organoid models have been developed for breast (90), colon (92), and prostate cancers(93). Recently, pancreas and pancreatic cancer organoids have also been developed (94-96).

The most comprehensive work in this domain currently utilizes 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 (97).

They performed deep genomic and transcriptomic analyses using both neoplastic and adjacent-normal organoids, 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 (98).

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 , suggesting that therapeutic testing of organoids will have clinical benefits.

4.1.2. regenerative medicine three-dimensional (3D) cell cultures

The use of 3D in vitro tumor models at the pre-clinical development stage, which simulate the in vivo physiological microenvironment, represents the intersection between tumor cell biology and tissue engineering. Such models can be useful in identifying potentially successful prototypes and eliminating failures at an early stage, thus bridging the gap between traditional monolayer cell culture and in vivo tumor cytology experiments. Consequently, an increasing number of tumor biologists have emphasized the importance of 3D tumor cell culture(99) .

Perche and colleagues evaluated the efficacy of chemotherapy drugs by constructing 3D globules of cancer cells. In this model, adriamycin was administered as a single drug or in combination with other anti-tumor drugs. The 3D structure of cancer cells limited drug permeability to the outer cell layer, indicating that globules have higher drug resistance than monolayer cells (100).

Similarly, Jung and colleagues constructed a 3D lung cancer model and studied the effects of Cisplatin and etoposide in standard chemotherapy regimens, providing important information to guide therapeutic approaches.

The 3D cultures are categorized as scaffold and scaffold -free techniques. Some of the components used in scaffold methods are Matrigel, hyaluronic acid, Polyethylene glycol (PEG), polyvinyl alcohol (PVA), polylactide-co-glycolide (PLG), polycaprolactone (PLA) (101-103). The scaffold-free techniques are based on suspending the cells utilizing hanging drops, rotating flasks and an agitation-based method (104). The current techniques of 3D culture in pancreatic cancer included organoids, spheroids, co-culture, and biotechnical microsystems. Here, we discuss all these methods in detail.

4.1.3. Organoid 3D cell cultures

Organoids can be defined as mini-organ-like clusters that are grown from stem or progenitor cells. These progenitor cells proliferate and differentiate to form multicellular and heterogeneous clusters that contain cell types with phenotypes similar to those of the original human organ from which the progenitor cells were derived. It is important to note that both spheroids and organoids can be either hollow or solid structures (105). Organoids are commonly grown by embedding them in a matrix or by culturing them in air-liquid-interface systems, although other methods such as spinner bioreactors have also been described (106, 107).

In cancer research, the term "organoid" has been expanded to include tumor-like cell clusters that are grown from tumor specimens (108, 109). Some publications refer to these tumor-derived multicellular clusters as "tumoroids" (110).

Because of the organoid's capacity to represent diverse areas of the body, organoids are becoming increasingly relevant in the research of genetic disorders(111, 112). Anticarcinogen treatment responses, particularly for targeted medicines, are heavily influenced by the genetic and epigenetic backgrounds of cancer patients(113, 114). Organoids offer a distinct advantage over standard monolayer culture techniques in that they are self-renewing and self-organizing structures, with unrivaled potential for a wide range of applications(115). Reliable, specialized in-vitro culture and analytical methodologies are required to properly employ these models in basic research, drug screening, and disease modeling. There are two significant distinctions between Organoids and Spheroids overall:

The basis of the driving factor behind their growth: Spheroids grow largely by cell-to-cell adhesion, whereas organoids are formed by internal developmental mechanisms.

The amount of time that 3D cultures may be sustained: In vitro, cell growth in culture requires an immature stem cell population to replenish dying cells over time. Organoids are made up of a population of stem cells that are maintained in vitro, ensuring their long-term survival. This is accomplished by optimizing culture conditions for growth, such as the use of a basement membrane matrix (i.e., Matrigel®) and the addition of a variety of agonists (e.g., Wnt and tyrosine kinase receptor) and inhibitors (e.g., bone morphogenetic protein/ transforming growth factor-)(116) .

4.1.4. Patient-Derived Tumor Organoids

The complex interactions between genetic alterations and niche factors during carcinogenesis have also been investigated using cancer organoid technology. Surgically resected/biopsied tissues and circulating tumor cells can also be used to create patient-derived cancer organoids (Figure 3). Patient-derived tumor organoids and spheroids (also known as tumor spheres) are simple to create and serve as reliable drug discovery and development methodologies(117).

Several advantages distinguish patient-derived tumor organoids from 2D monolayer and 3D spheroid cultures: (1) Patient-derived tumor organoids more closely replicate the patient's original tumor with a more diverse cell population; (2) Healthy tissue equivalents can be created, enabling side-by-side treatment response comparisons between the tumor and the healthy organoid from the same patient, which can be used to forecast a customized treatment window; (3) Developing patient-derived tumor organoids is less expensive than developing patient-derived xenograft (PDX) models since it takes less time and resources, and is more suited to high-throughput drug screening(117).

In the clinic, patient-derived tumor organoids have been proven to accurately predict patient medication response (118). However, one drawback of patient-derived tumor organoids is that they lack the interorgan connection found in more complicated in vivo systems, which can have an impact on tumor development as well as therapy response(119, 120). Efforts are now being made to improve organoid and immune cell co-culture procedures(121) .

5. Discussion

The in vivo model of pancreatic cancer is a widely used animal model to investigate the biology of pancreatic cancer and test potential treatments. This model involves the injection of pancreatic cancer cells into mice or rats, which are subsequently monitored for the development of pancreatic tumors. This approach enables researchers to study the growth and spread of cancer and to investigate the effects of genetic and environmental factors on the development and progression of pancreatic cancer (122, 123).

In recent years, 3D cell culture has emerged as a promising tool for modeling cancer, including pancreatic cancer(124). 3D cell culture utilizes a three-dimensional scaffold to support cell growth and proliferation in vitro. This approach enables researchers to study the behavior of cancer cells, including their response to drugs and other treatments, as well as interaction with the tumor microenvironment.

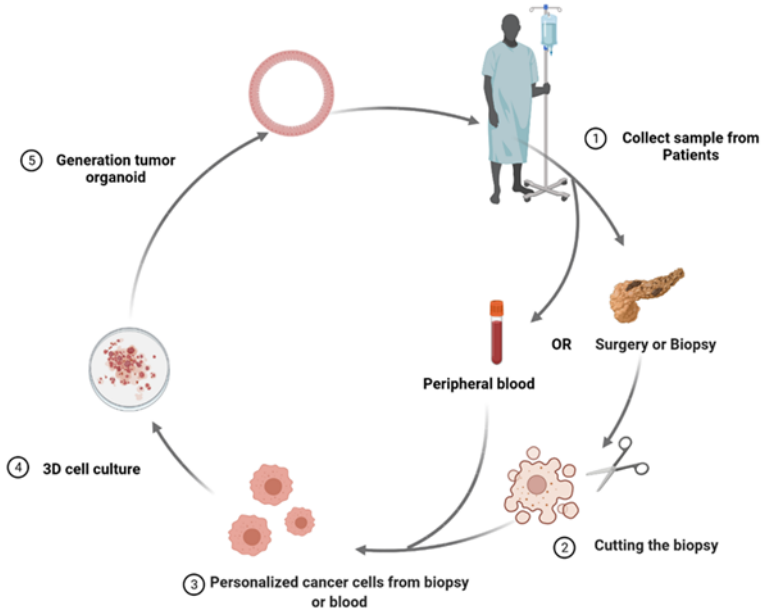

Figure3. Patient-derived cancer organoids

3D cell culture utilizes a three-dimensional scaffold to support cell growth and proliferation in vitro. This approach enables researchers to study the behavior of cancer cells, including their response to drugs and other treatments, as well as their interaction with the tumor microenvironment. Furthermore, 3D cell culture models can provide insights into the mechanisms of metastasis and invasion(125).

Organoids represent another in vitro model that has gained considerable attention in the study of cancer, including pancreatic cancer. Organoids are three-dimensional cell cultures that are derived from stem cells and mimic the structure and function of organs. This approach has been used to investigate the development and function of organs, as well as to study diseases. In the context of pancreatic cancer, organoids offer a valuable tool to investigate disease progression and treatment response, as well as to develop personalized treatments. Additionally, organoids can be used to investigate the effects of pancreatic cancer on other organs in the body (11).

In conclusion, although 3D cell culture has some limitations, it offers distinct advantages in the investigation of pancreatic ductal adenocarcinoma (PDAC) biology. These advantages include high stability, similarity to human PDAC cells, ability to predict disease progression and prognosis, and potential to develop personalized treatments by testing drug efficacy and resistance.

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