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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

Pancreas 3D Organoids: Current and Future Aspects as a Research Platform for Personalized Medicine in Pancreatic Cancer

Leticia Moreira,[1,2] Basil Bakir,[1] Priya Chatterji,[1] Zahra Dantes,[3] Maximilian Reichert,[3] and Anil K. Rustgi[1]

[1]Division of Gastroenterology, Departments of Medicine and Genetics, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania; [2]Department of Gastroenterology, Hospital Clínic, Centro de Investigación Biomédica en Red en Enfermedades Hepáticas y Digestivas, IDIBAPS, University of Barcelona, Catalonia, Spain; [3]II. Medizinische Klinik und Poliklinik, Klinikum Rechts der Isar, Technische Universität München, Munich, Germany

SUMMARY

An organoid is as a group of epithelial cells growing in a 3-dimensional structure, with self-renewal and self-organization capacities, which recapitulates the tissue of origin. Use of organoids as a powerful tool for investigation and as a step toward personalized medicine is reviewed.

Pancreatic ductal adenocarcinoma is one of the most aggressive forms of cancer, and the third leading cause of cancer-related mortality in the United States. Although important advances have been made in the last decade, the mortality rate of pancreatic ductal adenocarcinoma has not changed appreciably. This review summarizes a rapidly emerging model of pancreatic cancer research, focusing on 3-dimensional organoids as a powerful tool for several applications, but above all, representing a step toward personalized medicine. *(Cell Mol Gastroenterol Hepatol 2017;■:■–■; https://doi.org/10.1016/j.jcmgh.2017.12.004)*

Keywords: 3D Organoids; Pancreas; Adenocarcinoma; Personalized Medicine.

Pancreatic ductal adenocarcinoma (PDAC) is a rapidly progressing and usually fatal disease, and is the eighth leading cause of global cancer-related deaths in men and the ninth in women.[1] In the United States, PDAC is the third leading cause of cancer mortality and it is projected to be the second leading cause of cancer mortality by 2020.[2] According to the American Cancer Society, in 2016, a total of 53,670 people were diagnosed with PDAC in the United States and 43,090 died from this cancer. Pancreatic cancer accounts for about 3% of all cancers in the United States and for approximately 7% of cancer deaths. Although treatments have improved, especially in the fields of immunotherapy and adjuvant chemotherapy, PDAC has an average 5-year survival rate of only 7%–8%.[3]

The reasons for this morbid outcome are multifactorial, including diagnosis at late stages, rapid progression with metastasis to other organs, and resistance to conventional therapeutic modalities.[4] Most patients are diagnosed with metastases and succumb to disease within 6–12 months of diagnosis.[5] Therefore, understanding the mechanisms underlying disease initiation and progression are critical for early detection, risk stratification, and targeted therapeutic strategies.

The most frequent precursors of PDAC are microscopic pancreatic intraepithelial neoplasia (PanIN), followed by intrapapillary mucinous neoplasm and mucinous cystic neoplasm. PanINs are microscopic (<5 mm) mucinous-papillary lesions, which lead to invasive carcinoma.[6] Ductal adenocarcinomas display an intense stromal reaction that has been postulated to serve as a physical barrier to delivery of chemotherapy.[7] PDAC is associated with several gene alterations, such as activation of oncogenes (mutant *KRAS* is found in >90% of PDAC) and inactivation of tumor suppressor genes (*TP53*, *p16/CDKN2A*, *SMAD4*, and *BRCA2*).[8–10] Additionally, several genome-wide studies have been done. This has resulted in the identification of novel somatic mutations, although in low frequency, copy number variations, structural variations, and epigenetic alterations.[11–14] To that end, Bailey et al[11] performed a very elegant study where they combined the analyses of whole genomes, exomes, and RNA sequencing from 456 PDACs. Using this approach, they were able to define 4 different subtypes of PDAC (squamous, pancreatic progenitor, immunogenic, and aberrantly differentiated endocrine exocrine) and associated each subtype to specific molecular pathways, as well as histology and survival.

Despite these advances in the understanding of the mechanisms underlying PDAC pathogenesis, the impact on patient benefit is lagging. As a result, new model systems are being developed and used to fill this gap with the hope of translation into improved diagnostics and therapeutics.

Abbreviations used in this paper: 3D, 3-dimensional; GEMMs, genetically engineered mouse models; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; PDX, patient-derived tumor xenografts.

2352-345X
https://doi.org/10.1016/j.jcmgh.2017.12.004

This review covers the past and current model systems briefly, and then focuses on 3-dimensional (3D) organoids as a newer relevant model system.

Pancreatic Cancer Cell Lines

The first human pancreatic cancer cell line was generated and published in 1963,[15] and since then, many PDAC cell lines have been developed from murine and human tumors. The use of cell lines has many advantages: ease of use, minimal cost, and the ability for genetic and pharmacologic manipulation. Although cell lines have been very advantageous in pancreatic cancer research, this approach has several key limitations. First, many cell lines have been generated from metastatic tumors, and thus, primary PDAC and PanIN lesions are commonly underrepresented. Second, the lack of other cell types (fibroblasts, nerves, immune cells, adipocytes, endothelial cells) makes it difficult to reflect the physiological dynamics of the disease with accuracy. Furthermore, different expression profiles in the cell lines, as compared with the patient tumors or xenografts, have been reported,[16,17] with the potential selection of more aggressive clones during the generation of the cell lines.[18] Additionally, normal pancreatic ductal cells are challenging and difficult to maintain in culture, so the comparison between normal cells and tumor cells is often not possible. Finally, repeated passaging of cell lines inevitably results in genetic drift.

Xenografts

Xenograft models consist of implantation of pancreatic cancer cells either orthotopically or ectopically (usually subcutaneously) into immunodeficient mice, generating a tumor that might recapitulate a human tumor. Although this approach does offer a better representation of human tumors as compared with *in vitro* cell lines, problems with fidelity and therapeutic response has been reported.[19–21] One potential way of surmounting these limitations involves developing xenografts from patient tumors, which are known as patient-derived tumor xenografts (PDX). PDX helps recapitulate the genetic and phenotypic alterations of human tumors more effectively, which makes them a useful tool for determining drug response,[22] with a positive predictive value of more than 80%.[23] There are limitations with PDX, including the requirement for a large amount of tissue, expense, and time.[24]

Genetically Engineered Mouse Models

This model involves the use of genetic engineering techniques that can either activate or silence gene expression in mice to model disease characteristics. Genetically engineered mouse models (GEMMs) are an excellent approach to study disease progression from early stages (even PanIN) to primary/metastatic PDAC.[25] Some of the advantages in autochthonous models are that tumors develop spontaneously, interactions between the stroma and the tumor are maintained, tumor microenvironment dynamics are captured, and metastatic biology can be investigated. The use of GEMMs facilitates investigation of specific mutations under spatiotemporal control using the *Cre-LoxP* system, among other systems. The most frequently used mouse models take advantage of pancreas-specific cre-lines (eg, *Pdx1-Cre* or *Ptf1a-Cre*) in combination with a mutant allele of $Kras^{G12D}$ (which activates the gene driven by its endogenous promoter) and *TP53* loss.[26,27] This model phenocopies the human disease with high fidelity. More recently, the Saur group introduced the next-generation of GEMMs by combining 2 independent recombinase systems (*Cre-LoxP* and *Flp-FRT*), thereby allowing manipulation of distinct compartments (tumor and stroma) at specific time points within the organism. This allows investigators to ask specific questions regarding tumor-stroma interactions or to explore factors required for tumor maintenance.[28]

Although GEMMs are extremely valuable for understanding tumor biology, the breeding and maintenance of GEMMs are expensive and time consuming. Additionally, gene mutations are being introduced typically into the germline of the mouse, when they occur somatically in human tumors. Nonetheless, the use of next-generation mouse models and alternative approaches, such as unbiased genetic forward screens,[29] may allow for additional new insights.

Pancreatic Organotypic Model Systems

The ideal disease model should recapitulate the structure and genetic profile of the tissue under study, represent the heterogeneity and different stages of the disease, and respond to stimuli in a physiological manner. Moreover, from a practical point of view, this model needs to be feasible, reproducible, easy to maintain, and inexpensive. Therefore, even after recognizing the many strengths and applications of GEMMs, it is not a perfect model. Nonetheless, in recent years, there has been parallel attention to 3D cell culture and 3D organoid model systems.

Three-Dimensional Cell Culture

The 3D cell culture technique prevents cells from attaching to the bottom of the plate by maintaining the cells in suspension or embedding them in a matrix.[30] Initial attempts to establish 3D cultures from normal and tumor cells were unsuccessful, because of lack of cell viability and limited longevity.[31–33] However, in recent years, multiple laboratories, including ours, have developed 3D cultures of murine and human tissues, using slightly different conditions with specialized matrices to maintain cell-cell and cell-matrix interactions and polarized structures.[34–39]

3D cultures of epithelial cells give rise to spheres. Pancreatic spheres can be generated from pancreatic ductal and acinar cells.[40] Pancreatic spheroids may mimic some of the *in vivo* characteristics of PDAC, such as microenvironmental factors and drug responses.[37,41–43] 3D spheres from embryonic pancreatic cells resemble some aspects of pancreatic development, and display expression of PDX1 and SOX9.[44] Spheres generated from ductal cells have been used to study pancreatic carcinogenesis, especially the role of mutant *KRAS*, and with application to drug testing.[35,45–47]