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Organoid Models for Translational Pancreatic Cancer Research

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Abstract

Despite recent advances in the treatment of cancer, pancreatic ductal adenocarcinoma (PDAC) still retains the worst survival rate of common malignancies. Late diagnosis and lack of curative therapeutic options are the most pressing clinical problems for this disease. Therefore, there is a need for patient models and biomarkers that can be applied in the clinic to identify the most effective therapy for a patient. Pancreatic ductal organoids are ex-vivo models of PDAC that can be established from very small biopsies, enabling the study of localized, advanced and metastatic patients. Organoids models have been applied to pancreatic cancer research and offer a promising platform for precision medicine approaches.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest and most aggressive cancer types, with a five-year survival rate of less than 8% [1]. The poor outcomes of PDAC patients owe, in part, to the fact that the majority of patients (>70%) are diagnosed with advanced and metastatic disease, and are ineligible for surgical resection, the only potentially curative treatment [2, 3]. Even for patients diagnosed with localized tumors who are eligible for surgery, many patients' disease will recur after surgical intervention [4]. Most surgical patients are treated with systemic neoadjuvant or adjuvant cytotoxic chemotherapy [5–7*]. For those patients with locally advanced or metastatic disease, the standard-of-care treatments are the combination chemotherapy regimens Gemcitabine/nab-Paclitaxel [8] or FOLFIRINOX (5-Fluorouracil, Leucovorin, Irinotecan, Oxaliplatin) [9]. These cytotoxic regimens unfortunately do not cure the vast majority of advanced patients, and the median overall survival for these patients is less than one year [8, 9]. In addition, prolonged systemic treatment of chemotherapy is associated with toxicity in patients, leading to challenges in maintaining patients on these regimens. Furthermore, many patients have chemo-refractory disease, and there is currently no personalized approach for treatment selection for such patients.

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As the majority of PDAC patients do not present clinical symptoms until the cancer has advanced to the locally advanced or metastatic stage, there is a pressing need for clinically actionable, specific and sensitive early detection biomarkers for PDAC. Despite recent advances [10], CA19-9 remains one of the best biomarkers for assessing PDAC progression, but is not specific enough to be used for early detection [11]. One challenge for developing novel early detection strategies is the lack of normal, and pre-malignant models to identify cancer- specificity biomarkers.

Current precision medicine approaches to treating PDAC apply to a very limited subset of patients. For instance, while PDAC patients with mismatch repair deficiencies have been shown to respond to immunotherapy, only 1% of PDAC patients harbor alterations in these pathways [12, 13]. Similarly, patients with *BRCA2* and *KRAS*^{G12C} mutations have been found to benefit from targeted therapies, but these patients only constitute a few percent of all PDAC patients, and overall the genetic makeup of PDAC lacks actionable driver mutation [14, 15]. This highlights the need for both novel effective treatments for the majority of pancreatic cancer patients as well as precision medicine approaches to stratify each patient into the most effective treatment regime.

Patient-derived organoid models

Pancreatic cancer research has benefited from the availability of genetically engineered mouse models, monolayer cell lines, conditionally reprogrammed cells, patient derived xenografts, and more recently, three dimensional ex vivo culture systems (see reviews [16–18]). These 3D primary cultures are termed “organoids” in reference to the ability of such cultures to maintain cell types and architecture resembling the organ from which they were derived as well as their ability to regenerate tissue from that organoid when transplanted orthotopically [19]. While organoid culture systems were initially described to study healthy, non-transformed tissue, the technology has subsequently been applied to isolate and study tumors, including models of PDAC. For instance, the Clevers and Tuveson laboratories have utilized Wnt-ligand-rich conditions to stimulate indefinite propagation of pancreas normal and cancer organoids [19, 20**]. In contrast, the Muthuswamy and Skala laboratories propagate PDAC-derived organoids in medium that lacks Wnt ligands [21, 22]. More recently, the Sato laboratory identified Wnt-dependent and independent subpopulations of PDAC organoids highlighting the need for continuing refinements to organoid culture methods [23**]. Organoid culture methodology and protocols are readily available, enabling the research community to adopt the technology [24, 25].

Organoid models can be generated from surgically resected tumors as well as from the limited material present in small biopsies, such as fine needle aspirates [19, 20, 26]. In a large cohort study, the success rate of isolating and propagating PDAC organoids models was fairly similar (>70%) between resected tumor specimens and fine- needle biopsies, allowing organoid models to be generated from all stages of PDAC [20, 26]. These advanced culturing methods have enabled researchers to study cancer patients with localized disease as well as metastatic disease for many gastrointestinal tumors [20, 26–28]. The study of organoid cohorts representing a spectrum of tumor stages has led to important disease

progression findings such as the identification of enhancer programs that promote metastasis [29].

Genomics and transcriptomics of PDAC organoids

Studies of gastrointestinal organoids has not revealed the acquisition of new genomic alterations following extensive passaging in culture [30, 31]. For cancer patients, organoids faithfully capture the genomic alterations present in the tumors from which they were derived. Recently, multiple groups have performed targeted exome and whole genome sequencing to demonstrate good genomic concordance between primary PDAC tumors and their derived models [20, 32, 33]. While these studies generally demonstrate recapitulation of small nucleotide variants and structural variations in the organoids, thorough clonal analysis using single-cell or clonal organoid sequencing approaches remains unexplored for PDAC patients. Such clonal analysis performed on colorectal patient models yielded insight into organoid culture heterogeneity and its association with significant phenotypic consequences [34*]. Importantly, established organoid models of pancreatic cancer are predominantly composed of neoplastic cells enabling scientists to study low frequency nucleotide variants and copy number alterations that would be difficult to discern in primary tumor tissue with low neoplastic cellularity.

Molecular subtyping using transcriptome analyses of patient tumors has been described by multiple collaborative groups, and two major subtypes have been consistently identified [35–37]. One subtype has been described as a Basal-like, Squamous or Quasi-mesenchymal subtype which identifies PDAC patients with poor prognosis and is characterized by expression of *TP63* and other basal markers. In contrast, a second Classical or Pancreatic Progenitor subtype has been described, that is characterized by expression of ductal differentiation markers such as *GATA6* and identifies patients with a better prognosis [35–40]. Reassuringly, these subtypes were identified in independent cohorts of patient-derived organoids indicating that these transcriptional programs are maintained in ex-vivo cultures even in a Wnt-ligand- and growth factor-rich milieu [20, 23]. Seino and colleagues took advantage of the high neoplastic cellularity of the organoids to define functional subtypes of PDAC and demonstrated an inverse correlation between *GATA6* expression (associated with classical subtype) and strict requirement for WNT-signaling, thus postulating that *GATA6* acts as a master regulator of niche-dependency [23]. With inhibitors of the Wnt O-acetyltransferase Porcupine in clinical trials, this important finding highlights the need for precision approaches for patient selection when considering therapeutics approaches to the Wnt pathway.

Therapeutic testing of PDAC organoids

Ex-vivo cellular models of cancer, such as monolayer cell lines, are commonly used to test therapeutic approaches. However, translation of these studies into clinical benefit has been challenging [41*]. Patient-derived organoid cultures are an attractive model for therapeutic studies. As discussed above, organoids can be isolated from patients at all stages of pancreas cancer progression with as little starting tissue as the limited tissue available in a fine needle tumor biopsy. Secondly, organoids offer a pure neoplastic population of cells enabling study

of cancer-intrinsic sensitivities and resistances. Finally, beyond the scope of basic research, organoids could potentially serve as a personalized medicine platform (Figure 1). Using a large cohort of patient-derived organoids, Tiriac and colleagues have established a robust platform for testing single agent chemotherapy and targeted agents [20]. They demonstrate, in retrospective case studies, that organoid response to therapeutic testing, termed “pharmacotyping,” parallels patient sensitivity to chemotherapy. Through correlation of the drug sensitivity profile and the transcriptome of each organoid in the cohort, the group identified transcriptomic signatures of chemo-sensitivity. These RNA signatures were predictive of clinical outcome in adjuvant- and neoadjuvant- treated cohorts of PDAC patients. Other researchers have established similar platforms to test targeted and combination therapies, and these groups also found concordance between matched patient-derived PDAC organoids and patient sensitivity to treatment [21, 33]. Similar comparisons of organoid response to patient treatment response have been conducted in other gastrointestinal malignancies such as colorectal, intestinal and liver cancers demonstrating the robustness of this approach [28, 42, 43]. High-throughput drug screening of organoids requires either the adaptation of 3D culture methods to existing automation systems or the development of new automation systems designed to work with 3D cultures, but has the potential to discover transformative treatment strategies [44]. Clonal heterogeneity in pancreas cancer organoids remains to be explored and could be an underlying factor leading to chemo-refractory disease in patients. In colorectal cancer organoids, Roerink and colleagues have shown that clonal organoid cultures derived from single cells from a patient tumor can display heterogeneous responses to drug treatment [34]. In repeat biopsy-derived organoids taken over multiple years in a metastatic PDAC patient, the Tuveson laboratory has shown increased organoid resistance to chemotherapy coinciding with treatment refractory disease [20]. which could be due in part to clonal selection. These important finding should be a focus of future studies of pancreas cancer organoids, as they may affect how clinical testing of organoids is conducted in the future.

Patient-derived organoid therapeutic testing platforms warrant further evaluation in both retrospective and prospective clinical trials to demonstrate efficacy and, importantly, usability in a clinical setting where patients are diagnosed late and must rapidly begin therapy. Organoid isolation, expansion and testing currently requires weeks of effort in a specialized laboratory environment. To effectively couple organoid testing to the clinical care of advanced and metastatic patients, researchers must first refine their methodologies to accelerate testing of valuable compounds. Establishing the reproducibility and sensitivity of pharmacotyping platforms will be key to success, while establishing a clear threshold of drug-sensitivity will be needed to make assay results easily translatable for patient care. Finally, the identification of biomarkers of sensitivity or resistance using organoids provides additional tools for personalized medicine that may alleviate the requirement to establish an organoid model prior to the selection of initial therapies.

Co-culture models

Pancreatic ductal adenocarcinoma is characterized by a desmoplastic reaction resulting in dense, fibrotic stroma [45]. Monocultures of cancer organoids, while useful for determining cancer-intrinsic sensitivities, miss these important stromal cues. To address this issue,

Ohlund and colleagues set out to establish co-cultures of organoids and the resident fibroblasts of the pancreas, pancreatic stellate cells. They identified a minimal set of culture conditions in which the PDAC organoids and fibroblasts were mutually supportive to one another such that the co-cultures, but not mono-cultures, could survive and thrive [46**]. Using this system they identified two novel sub-populations of fibroblasts present in the PDAC microenvironment that may act in distinctive tumor-supportive and tumor-restrictive roles. In a separate study, Biffi and colleagues uncovered IL-1 and TGF-beta as PDAC-derived signals underlying fibroblast heterogeneity [47]. These results suggest novel approaches for treating PDAC by blocking tumor-supportive and promoting tumor-restraining fibroblasts. In parallel, Tsai and colleagues established a patient-matched triple co-culture system which includes PDAC organoids, cancer-associated fibroblasts and T-cells [48]. Inclusion of immune cells in the ex-vivo culture systems is a critical step to establish a platform for the study of immunotherapy in pancreas cancer. With a robust patient-matched co-culture system, researchers and clinicians will be able to assess various immunotherapy strategies [49] prior to patient administration.

Future Research and clinical outlook

Dissemination of organoid methodology has greatly stimulated pancreatic cancer research and already culminated in important findings, some described in this review, which may directly impact clinical care. Pharmacotyping is clearly an important area for clinical evaluation, and current methodology limitations will have to be overcome for pharmacotyping to benefit most patients. Biomarker discovery for treatment selection, should be prioritized, and organoids offer an ideal setting for such studies. The availability of published protocols, advanced organoid training courses, and commercially available reagents are making the organoid system approachable and accessible to the research community. Recent refinements in culturing conditions such as the elimination of animal serum from the organoid medium [50, 51], and development of defined synthetic matrices [52] are important advances that are essential for establishing reliable clinical assays. Current efforts to create an organoid repository such as the NIH/NCI supported Human Cancer Models Initiative will greatly benefit the research community by making validated models and protocols [53] easily available. Novel protocols which enable neuroendocrine differentiation of pancreatic ductal organoids could lead to advances in regenerative medicine [54, 55]. These findings are important for the many patients, including pancreatic cancer patients, who suffer from pancreatic endocrine insufficiency. As researchers have made dramatic breakthroughs using organoid models, care must be taken to translate these important findings to the clinic through rigorously conducted and controlled clinical trials.

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special interest (*)

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Figure 1:

Patient-derived organoids and stromal cells, such as immune cells and cancer associated fibroblasts, can be isolated from small and large tissue samples. Organoids cultures can recapitulate clonal heterogeneity of the neoplastic tissue. the Molecular (DNA/RNA) and phenotypic (drug testing) characterization of mono- and co-culture systems can provide actionable clinical insights for cancer patients at all stages of the disease.