Robust Establishment And Expansion Of Human Pancreatic Duct Organoids

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INTRODUCTION

The pancreas is essential for the maintenance of blood glucose levels and the production of digestive enzymes. The exocrine pancreas is composed of acinar cells that secrete the digestive enzymes and ductal cells that secrete a fluid rich in bicarbonate and line the ducts that transport the secreted enzymes to the duodenum. The exocrine pancreas is affected by severe pathologies such as acute and chronic pancreatitis, cystic fibrosis, and pancreatic cancer, with pancreatic ductal adenocarcinoma (PDAC) representing the most commonly found subtype of pancreatic cancer.

Studies of exocrine pancreas development and disease progression have largely been restricted to mouse models and patient-derived xenografts. Organoids provide a novel in vitro culture system that promotes the growth of primary and pluripotent stem cell (PSC)-derived cells in three-dimensional (3D) culture to generate structures that have morphologies that recapitulate the exocrine pancreas in vivo. Publications by Boj et al. and Broutier et al. have demonstrated that adult tissue-derived pancreatic organoids are composed of ductal and self-renewing progenitor-like cells, and have morphologies and gene expression profiles that mirror the tissue of origin, and that organoids derived from pancreatic tumors recapitulate many of the features of the parental tumor. As a result, pancreatic duct organoids are becoming a versatile tool for studying human pancreatic development, cell function, cell toxicity, drug efficacy, and disease progression.

To standardize and simplify the culture of human pancreatic duct organoids, we have developed PancreaCult™ Organoid Medium Kits (Human) for the robust establishment and expansion of pancreatic duct organoids.

METHODS

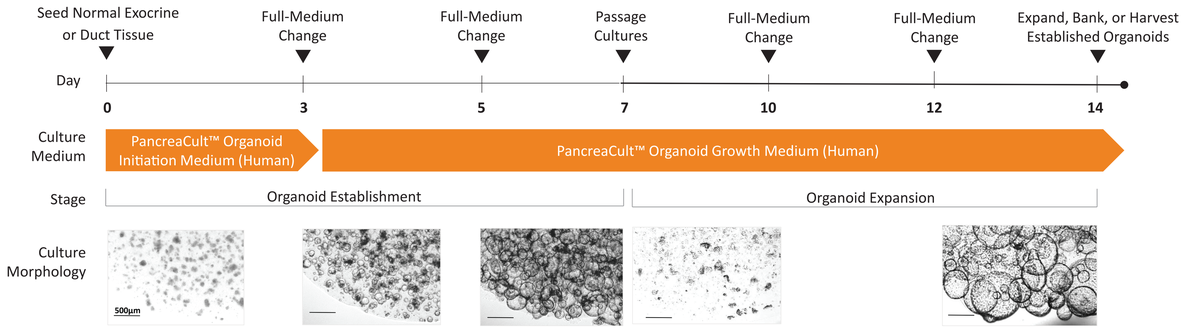

FIGURE 1. PancreaCult™ Human Enables Initiation and Expansion of Pancreatic Duct Organoids

Human pancreatic duct organoids are initiated in PancreaCult™ OIM for the first 3 days of culture, then switched to PancreaCult™ OGM for the remainder of the culture. Alternatively, organoids can be grown in PancreaCult™ OGM for a completely serum-free protocol, however, proliferative pancreatic ductal cells are better supported in PancreaCult™ OIM during the first 3 days of culture. Cultures should be passaged after 7 days with further medium changes every 2 - 3 days. Pancreatic ductal organoids are suitable for experimentation or banking after one full passage in PancreaCult™ OGM. Scale bars = 500 µm

RESULTS

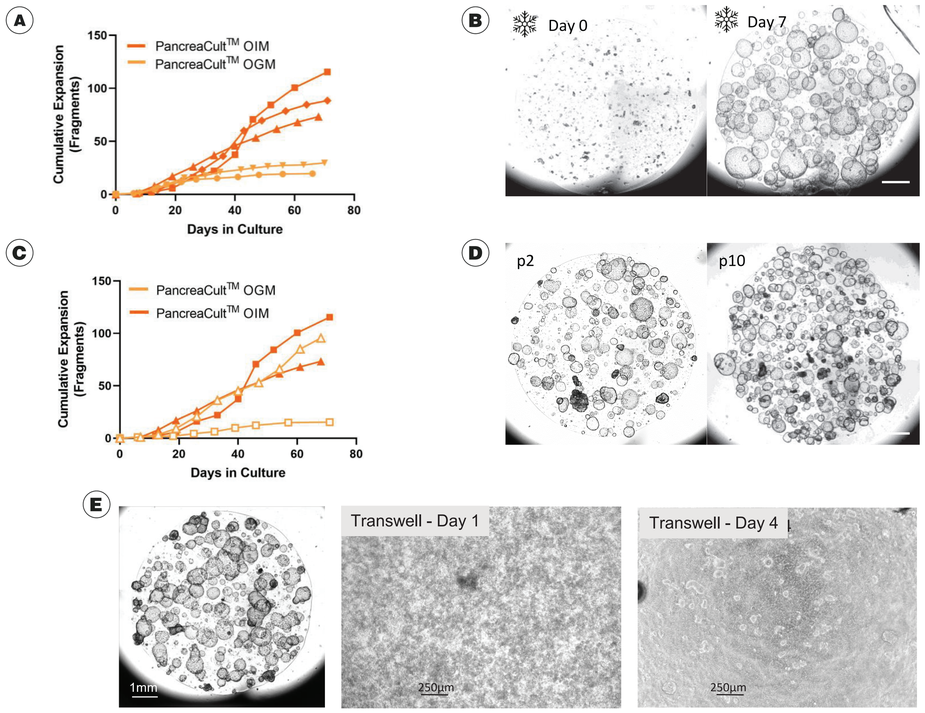

FIGURE 2. Robust Establishment, Maintenance, Thawing and 2D Re-seeding of Organoids in PancreaCult™ Human

(A) PancreaCult™ OIM/OGM (▲◆■) and OGM alone (● and ▼) can establish and maintain organoids for at least 10 passages (n=5). Expansion rates are donor dependent. **(B)** Organoids cryopreserved as fragments at p2 are quickly re-established 7 days post-thaw in PancreaCult™ OGM. ❄ = Cultures established from cryopreserved organoid fragments. **(C)** Establishment and maintenance of organoids from low viability tissue was improved by addition of PancreaCult™ OIM (■) but is not obligatory for all donors (△). **(D)** Morphology of organoid cultures established from low viability tissue in PancreaCult™ OIM and maintained in PancreaCult™ OGM Scale bars = 1 mm. **(E)** Organoids established and maintained until p4 in PancreaCult™ OGM were incubated 24h with 10% FBS (left). Organoids were dissociated and seeded onto a Matrigel-coated Transwell® insert in PancreaCult™ OGM supplemented with 10% FBS + ROCK inhibitor (Y-27632) and without PGE2 (middle). After 4 days cells formed a confluent monolayer (right).

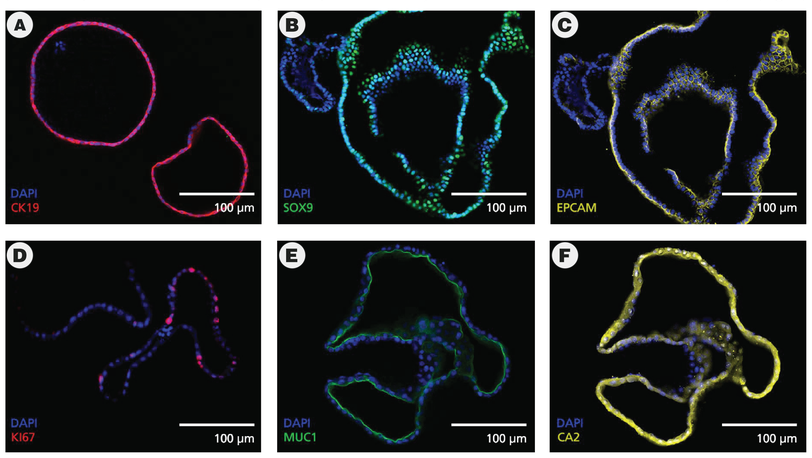

FIGURE 3. Pancreatic Duct Organoids Display Features of the Pancreatic Ductal Epithelium

Organoids grown using the PancreaCult™ Human display marker expression consistent with the pancreatic ductal epithelium when imaged using immunocytochemistry. Shown are organoids grown in PancreaCult™ OGM and stained for **(A)** pancreatic ductal marker CK19, **(B)** pancreatic ductal marker SOX9, **(C)** epithelial marker EPCAM, **(D)** proliferation marker KI67, **(E)** apical pancreatic duct marker MUC1, and **(F)** pancreatic ductal marker CA2. Organoids were imaged on passage 2 **(A)**, passage 3 **(B, C)** or passage 10 **(D-F)**.

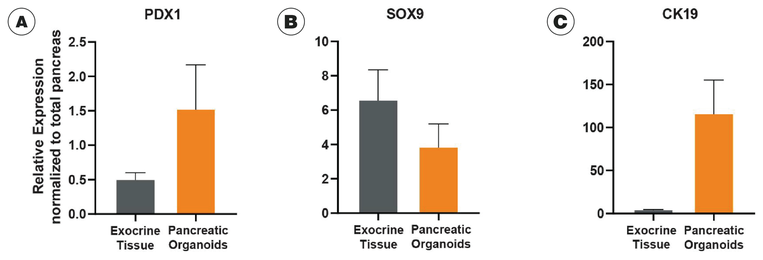

FIGURE 4. Pancreatic Duct Organoids Cultured in PancreaCult™ Human Show Pancreatic Marker Expression Levels Similar to Exocrine Tissue

Pancreatic duct organoids grown using the PancreaCult™ Human show marker expression levels similar to those observed in exocrine tissue. Analysis by qPCR showed pancreatic duct organoids were enriched for **(A)** PDX1 **(C)** CK19 and **(F)** LGR5 as compared to total pancreatic tissue, demonstrating enrichment of proliferative duct organoids. Comparable expression of **(B)** SOX9, **(D)** cystic fibrosis transmembrane receptor (CFTR), and **(E)** CA2 was observed in pancreatic duct organoids. Expression levels are normalized to TBP and UBC housekeeping genes (ΔCT) and total pancreas for relative expression levels (ΔΔCT).

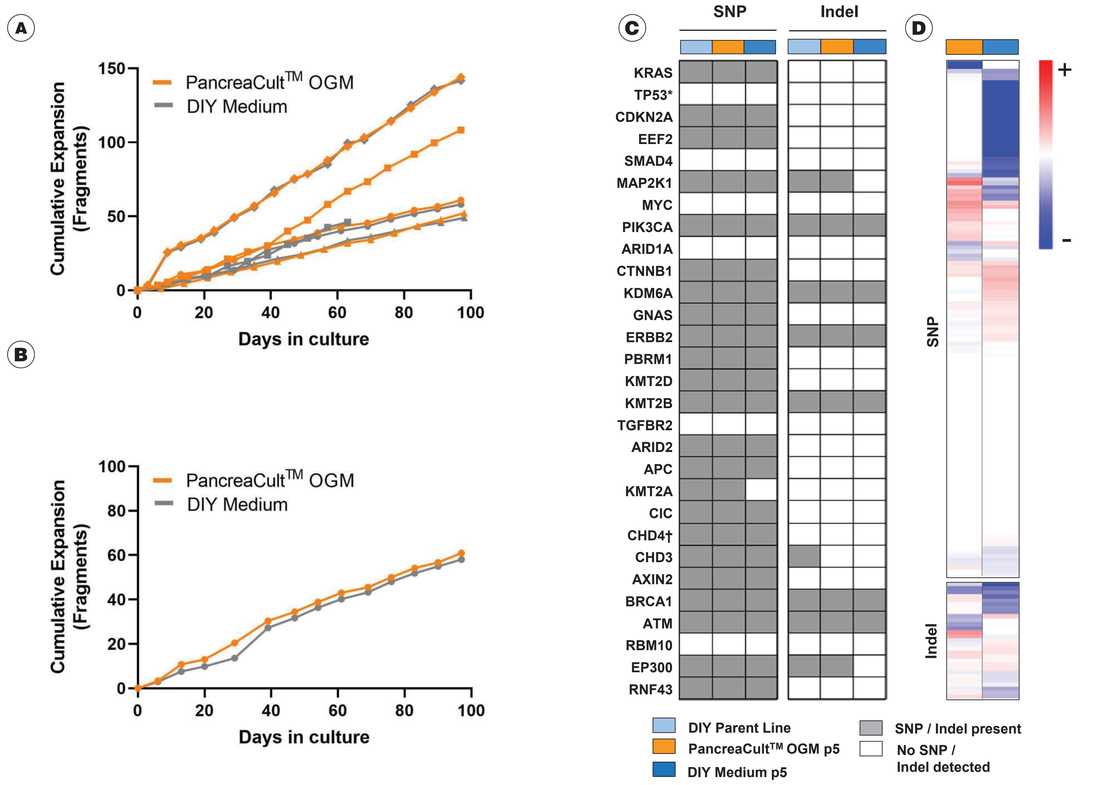

FIGURE 5. PancreaCult™ OGM Maintains the Growth and Mutational Profile of Pre-established PDAC Organoid Lines-

(A) Pre-established PDAC organoid lines maintained in PancreaCult™ OGM for at least 10 passages (n = 4). **(B)** Growth characteristics of PDAC line used for whole-exome sequencing analysis after culture in PancreaCult™ OGM and DIY medium. **(C+D)** WES was used to compare the presence of somatic SNPs and Indels in 29 oncogenic driver and repression genes for the PDAC Line depicted in Figure 5B. Sequencing was performed on samples collected before (light blue) and after 5 passages in PancreaCult™ OGM (orange) or the DIY Medium the line was established in (dark blue). Reads were aligned to GRCh38 with HISAT2 (v2.0.5). VarScan2 (v2.3.9) was then used in pairwise mode to classify variants identified by samtools mpileup (v1.15) into "new", "LOH" or "inherited" in relation to the starting cultures. **(C)** Grey and white boxes indicate the overall presence or absence of SNPs or Indels in the indicated genes compared to the parent line. *TP53 reads were undetectable indicating deletion, †Presence of SNP in CHD4 in the PancreaCult™ OGM cultured sample could be manually validated. **(D)** Percent of reads with a variant allele (SNPs and Indels) detected in the listed genes after 5 passages in PancreaCult™ OGM (left column) or DIY Medium (right column). Values are depicted as percent of reads relative to the parent line. Red = increased, blue = decreased or below detection limit of 6 reads. No losses of variants were detected in either culture medium, with a loss being defined as ≥ 20% of total reads in the parent line being a variant and 0% in daughter line with a minimum coverage of reads per region.

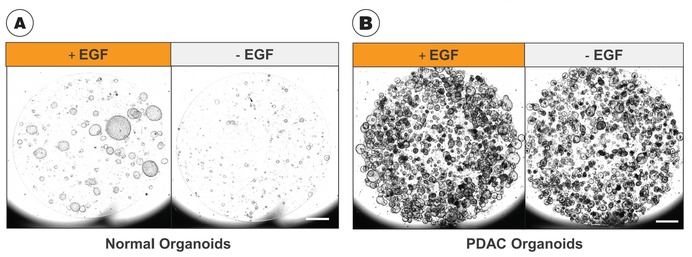

FIGURE 6. Removal of Epidermal Growth Factor (EGF) Allows for the Selection Against Normal Cells but Maintains Growth in Organoids derived from Dissociated PDAC Tumor Cells in PancreaCult™ Human

(A) EGF removal efficiently suppresses normal pancreatic duct organoid growth after 1 passage.
(B) Organoids established from cryopreserved dissociated cells (DTCs) in PancreaCult™ Human were expanded for 8 passages in low-oxygen culture (5%) in PancreaCult™ OGM. EGF removal over 2 passages maintained organoid growth, indicating the presence of KRAS-activated tumor cells. Scale bar = 500 µm.

Summary

- PancreaCult™ Human can establish and maintain long-term expansion of fresh and cryopreserved normal human pancreatic duct tissue
- PancreaCult™ OIM supports the culture establishment from low quality starting material
- Cryopreserved organoid fragments can quickly re-establish organoid cultures and organoids can be dissociated and re-seeded into 2D Transwell® cultures
- PancreaCult™ OGM maintains robust expansion and the genetic profile of pre-established PDAC organoids
- EGF removal can be used to suppress normal cell growth
- PancreaCult™ Human successfully established and expanded EGF-dependent organoids from dissociated PDAC tumor cells

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Acknowledgements

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