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 several 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 pancreatic 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 Beij et al. and Brouder et al. have demonstrated that adult tissue-derived 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**

To pre-established PDAC organoid lines maintained in PancreaCult™ OGM for at least 10 passages (n = 4). (B) Growth characteristics of PDAC organoids cultured in 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 lines depicted in Figure 5A. 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). Peaked aligned to GRCh38 with HISAT2 (v2.1.0), VarScan (v2.3.9) was then used in paired mode to classify variants identified by samtools mpileup (v1.15) into “new,” “LOH” or “inherited” in relation to the starting cultures. (E) Grey and white boxes indicate the overall presence or absence of SNPs or Indels in the indicated genes compared to the parent line. Each “x” indicates a undetectable, indicating deletion. Presence of SNP in CHD4 in the PancreaCult™ OGM cultured sample could be manually validated. (F) 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 allele (SNPs and Indels) detected in the listed genes after 5 passages in PancreaCult™ OGM (left column) or DIY Medium (right column). 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 allele (SNPs and Indels) detected in the listed genes after 5 passages in PancreaCult™ OGM (left column) or DIY Medium (right column).

**RESULTS**

Pancreatic duct 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, D) or passage 10 (D, E, 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) PDF1, (B) CX19 and (F) LGCR as compared to total pancreatic tissue, demonstrating enrichment of proiferative 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 U6B housekeeping genes (ΔΔCT) 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 organoids cultured 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 lines depicted in Figure 5A. 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). Peaked aligned to GRCh38 with HISAT2 (v2.1.0), VarScan (v2.3.9) was then used in paired mode to classify variants identified by samtools mpileup (v1.15) into “new,” “LOH” or “inherited” in relation to the starting cultures. (E) Grey and white boxes indicate the overall presence or absence of SNPs or Indels in the indicated genes compared to the parent line. Each “x” indicates a 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 allele (SNPs and Indels) detected in the listed genes after 5 passages in PancreaCult™ OGM (left column) or DIY Medium (right column). 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 allele (SNPs and Indels) detected in the listed genes after 5 passages in PancreaCult™ OGM (left column) or DIY Medium (right column).

**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.