Unlocking Pancreatic Disease Solutions: Human iPSC-Derived Ductal Organoids for Cutting-Edge Drug Discovery and Functional Analysis

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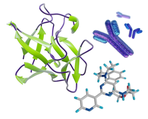
Introduction

Robust human in vitro models that enable functional evaluation of new therapies are critical for advancing treatments for pancreatic ductal diseases, including cystic fibrosis, pancreatitis, and pancreatic ductal adenocarcinoma. Human induced pluripotent stem cell (hiPSC)-derived pancreatic ductal organoids (PDLO) offer a physiologically relevant platform for modeling ductal epithelial biology and assessing therapeutic strategies aimed at restoring or modifying gene function. Here we report on the generation and comprehensive cellular identity, structural, and functional characterization of human iPSC-derived PDLOs as a scalable preclinical model to support drug safety and efficacy testing.

Materials and Methods

Human iPSCs were differentiated into PDLOs using a 30-day stepwise protocol[1]. Cells progressed through definitive endoderm and pancreatic progenitor stages in 2D culture prior to transition into 3D basement membrane-based organoid culture. Differentiation efficiency and lineage commitment were assessed at defined stages using brightfield microscopy and flow cytometry. Protein expression of progenitor and mature ductal markers were quantified using automated capillary-based immunoassays. Organoid architecture and epithelial polarity were evaluated by immunofluorescent staining. Functional characterization was performed using cystic fibrosis transmembrane conductance regulator (CFTR) activity assay based on forskolin-induced organoid swelling and pharmacological CFTR inhibition.



R&D Systems Reagent Portfolio

Stage-specific expansion, differentiation, and characterization of human iPSC-derived cells were performed using R&D Systems growth factors, small molecules, matrices, supplements, and antibodies.

Automated Capillary-Based Immunoassays using Simple Western™ Technology

Protein expression of key lineage markers was assessed using the Leo™ System powered by Simple Western technology, providing automated, high-throughput, and quantitative analysis with minimal sample input.

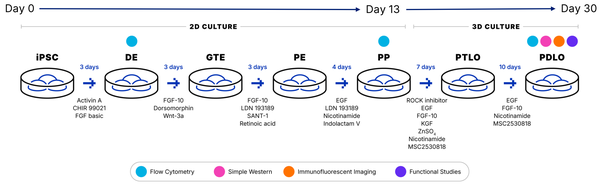


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Figure 1. Scalable workflow for the generation of the hiPSC-derived PDLO model. Human iPSCs were differentiated over 30 days through a stepwise process progressing from definitive endoderm (DE) to gut tube endoderm (GTE) to pancreatic endoderm (PE) to pancreatic progenitors (PP) in 2D culture. On day 13, pancreatic progenitors were reseeded into 3D culture to support further differentiation to pancreatic trunk-like organoids (PTLO) and ultimately pancreatic ductal organoids (PDLO). Quality control was performed throughout the differentiation to monitor lineage specification. At the end of the workflow, PDLOs were validated at the protein, structural, and functional levels.

Results

Differentiation of hiPSCs to Pancreatic Ductal Organoids

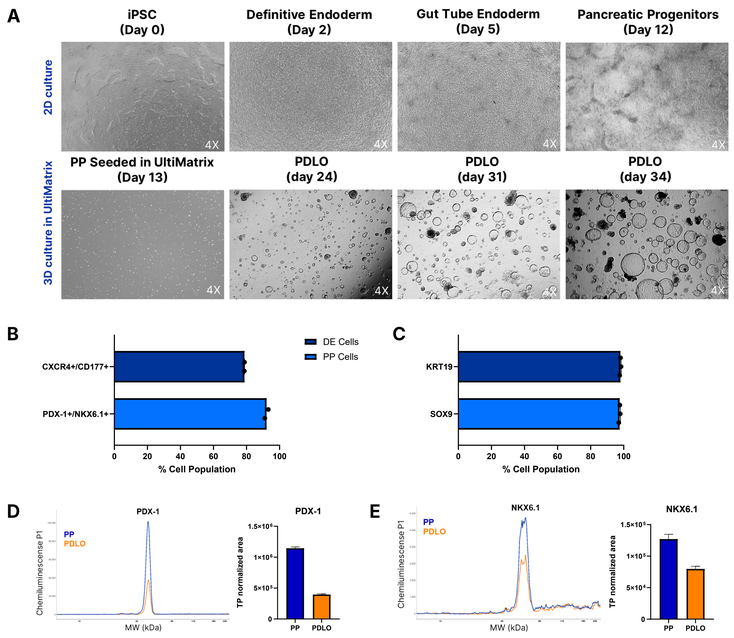

Figure 2. Cells exhibit the expected morphologies and lineage characteristics throughout the differentiation. (A) Representative brightfield images show cell morphology over the 30-day differentiation workflow. Images via EVOS Cell Imaging System. **(B)** Definitive endoderm cells were stained with anti-CXCR4 and anti-CD117 antibodies. Pancreatic progenitor cells were stained with anti-PDX-1 and anti-NKX6.1 antibodies. All samples were quantified by flow cytometry. Data are presented as the mean of two biological replicates. **(C)** PDLOs were stained with anti-KRT19 and anti-SOX9 antibodies and analyzed by flow cytometry. Data are presented as the mean of three biological replicates. **(D)-(E)** Cell lysates collected from day 13 pancreatic progenitors and day 28 PDLOs were examined for expression of the progenitor markers **(D)** PDX-1 and **(E)** NKX6.1 using Simple Western analysis. Representative electropherograms are shown, with marker expression quantified by measuring the area of each peak and normalizing to total protein (TP normalized area). Data are presented as the mean of three technical replicates.

Characterization of hiPSC-Derived PDLO Cell Identity

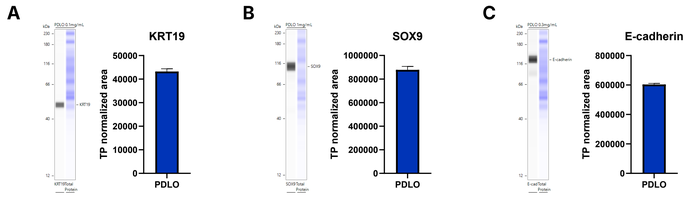

Figure 3. Validation of the PDLO model by Simple Western analysis. Cell lysates collected from day 28 PDLOs were examined for the expression of duct-specific proteins **(A)** KRT19, **(B)** SOX9, and **(C)** the structural marker E-cadherin using Simple Western analysis. Representative lane views are shown for each protein alongside total protein expression. Marker expression was quantified by measuring the area of each peak and normalizing to total protein (TP normalized area). Data are presented as the mean of three technical replicates.

Characterization of hiPSC-Derived PDLO Structure and Function

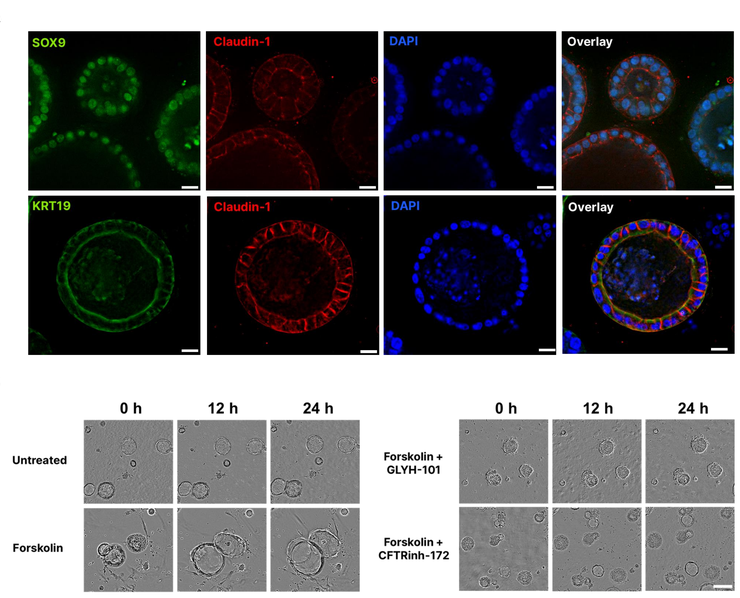

Figure 4. Evaluation of the PDLO model architecture and function. (A) hiPSC-derived PDLOs were fixed, cryo-sectioned, and stained for KRT19, SOX9, and Claudin-1. Images via Leica THUNDER Imaging System. Scale bar: 200 µm. **(B)** Function of the PDLO model was evaluated using a forskolin-induced-swelling assay. Organoid swelling was observed within twelve hours of forskolin treatment. Co-treatment with CFTR inhibitors, GlyH 101 or $CFTR_{Inh}$172, effectively blocked the swelling response. Images via Incucyte Live-Cell Analysis System. Scale bar: 200 µm.

Conclusions

- We demonstrate the efficient generation of human iPSC-derived pancreatic duct-like organoids with expected cell identity (>90% KRT19+ and SOX9+ cells), organized epithelial architecture, and robust ductal function.
- The presence of functional, inhibitor-responsive CFTR activity establishes this model as a relevant platform for evaluating gene replacement, gene editing, and other therapeutic strategies targeting pancreatic ductal disorders.
- Combined with GMP-grade reagents and the automated analytical platform, these PDLOs provide a scalable, standardized, and clinically relevant in vitro platform to support preclinical development and functional validation of advanced therapies

References:
1. Breunig, M., et al., STAR Protocols. 2021.