Development and Characterization of a Patient-Derived Liver Organoid Biobank

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Abstract

Background and Purpose: The liver, as the largest solid internal organ, is responsible for numerous essential functions. In addition, liver diseases account for about 2million deaths annually worldwide (1). Traditional models for studying liver biology, such as 2D cultured primary cells, 3D spheroids and cell lines, do not accurately represent the in vivo metabolic and structural complexities. Additionally, animal models are often ineffective, costly, time-consuming and associated with ethical concerns. Thus, there is a critical need for advanced, tissue-mimicking liver models. Organoids are self-organizing 3D-cultured structures that replicate the structure and functionality of the original tissue (2). Patient-derived organoids (PDOs) closely resemble the source tissue and may mimic individual responses to therapies. In this study, we report the development and characterization of a biobank comprising 11 individual liver PDO lines, reflecting donor variability and enhancing the relevance of these models for toxicology applications.

Methods: Liver tissue were obtained with donor consent. PDOs were generated by modifying a previously published protocol (3). In brief, samples were minced, digested, filtered, mixed with Matrigel (Corning) and cultured in liver PDO-specific growth media. When appropriate, undifferentiated (immature) PDOs were differentiated (matured) using media tailored for this purpose.

Results: PDO lines were successfully expanded and cryopreserved. The immature PDOs could be thawed and further expanded using our standard operating procedures, confirming the robustness of these lines. All PDO lines expressed liver-specific biomarkers, as confirmed by qPCR and confocal microscopy. When matured, these lines showed increased albumin and urea production, as well as inducible cytochrome P450 (CYP) activity, confirming their functional maturity. Levels of these phenotypes were donor dependent, suggesting that the biobank could represent the patient pool. Each PDO line was also established as microbial and viral contamination free and confirmed as unique through short tandem repeat analysis.

Conclusions: This liver PDO biobank provides a representative snapshot of patient populations and may serve as a valuable tool for pharmaceutical clients. To date, we have successfully established and characterized 11 liver PDO lines, with further characterization ongoing. Our liver PDOs will significantly enhance the understanding ofliver biology and disease mechanisms, offering substantial applications in drug testing, including DMPK and ADME/Tox studies. Future efforts will focus on scaling up, developing liver-specific assays, and generating PDOs from patients with specific liver conditions, such as hepatocellular carcinoma, MASLD, and NASH.

Introduction

- ❑ Liver is the largest solid internal organ and holds 13% of the body's blood supply
- ❑ It has 2 main lobes, with ~1000 lobules. The lobules connect to ducts to form common hepatic duct.
- ❑ Common hepatic duct transports bile to gallbladder and duodenum via the common bile duct
- ❑ Liver performs over 500 key physiological functions:
  - Production of albumin, bile, cholesterol and some plasma proteins
  - Metabolism of food, drug and foreign materials
  - Conversion of excess glucose into glycogen
  - Processing of hemoglobin
  - Conversion of ammonia to urea
- ❑ 2D cultures do not recapitulate cell-cell and cell- ECM interactions and demonstrate altered cell signaling.
- ❑ Animal models are fundamentally different and patient-derived xenografts take very long to grow and are not reproducible on a large scale.
- ❑ 3D-cultured organoids show structural and functional similarity to the human liver. These structures represent the spatial, "*in vivo*-like" organization of multiple cell types.
- ❑ Human organoid models are increasingly recognized as **New Approach Methodologies (NAMs)**. The FDA encourages integration of NAMS to improve translational predictivity and reduce reliance on animal models in preclinical testing.

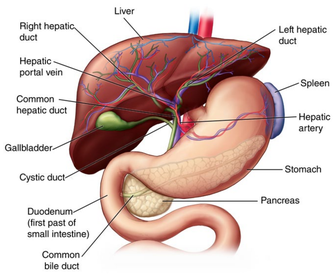

Fig. 1. Anatomy of the liver. www.hopkinsmedicine.org/health/conditions-and-diseases/liver-anatomy-and-functions

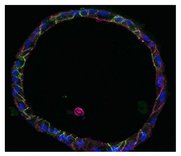

Fig. 2. Liver PDO. Staining of liver PDO SCC580 with MilliporeSigma anti-E-Cadherin, ZRB1692 (Monoclonal Ab, clone 1G14 (green). Nucleus stained with DAPI (blue), F-Actin stained with phalloidin (magenta).

Purpose

- ❑ The purpose of this work is to develop a multi-donor, healthy liver organoid biobank.
- ❑ These PDO are generated from mature liver, and therefore may retain adult tissue characteristics and hence considered immature, but partially differentiated
- ❑ We offer the healthy PDO lines as part of a biobank or as individual lines
- ❑ Whenever needed, these immature PDO lines (ILO) may be differentiated into mature hepatocyte-rich, matured PDO (MLO)
- ❑ MLO may be used in drug metabolism, drug transport and toxicity assays
- ❑ Our liver PDO biobank has high potential use in personalized medicine especially when coupled with genome editing technologies
- ❑ Strong demand for such liver models in both biopharmaceutical industry and academia, especially with the **FDA Modernization Act 2.0** that supports the use of NAMs in drug development

Sourcing

Tissue sourced via Scientist.com

- ❑ Liver tissue sourced ethically with Informed Patient Consent
- ❑ Liver tissue was either (1) cryopreserved tissue: excised→ slow frozen using cryopreservative (SOP developed in-house)→ delivered on dry ice OR

(2) fresh tissue: excised→ placed in University of Wisconsin media with Primocin (100 µg/ml) → Delivered within 24 h of collection.

Workflow: Generating partially differentiated PDO

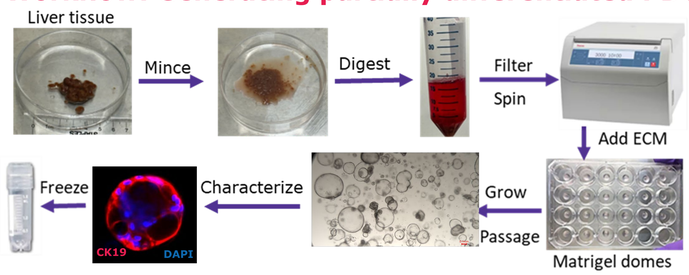

Fig. 3. Generating liver PDO from vendor-sourced tissue. Sourced liver tissue was minced, digested and filtered. The filtrate was centrifuged, suspended in Matrigel® (Corning) and plated. PDO were cultured in liver PDO expansion media (generated in-house), passaged at specific time points and cryopreserved when appropriate. Scale bars, 100 µm.

Liver PDO show consistent growth

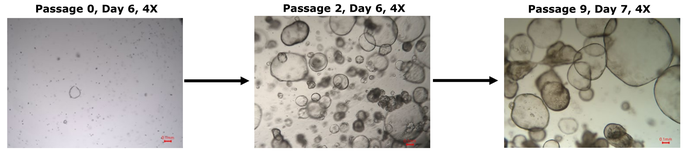

Fig. 4. Robust process standardized for generating liver PDO. Human liver PDO (representative line, Cat. No. SCC570) was generated from cryopreserved tissue; and passaged over time. PDO could be expanded and showed robust and consistent growth with passages. Scale bars, 100 µm.

Liver PDO show robust growth following banking

Fig. 5. Cryopreserved liver PDO showed robust growth post thaw. Human liver PDO (representative line, Cat. No. SCC570) was cryopreserved. Liver PDO could be successfully expanded following thaw and showed robust growth, following in-house protocols.

Liver PDO contain multiple cell types, are polarized and bipotential

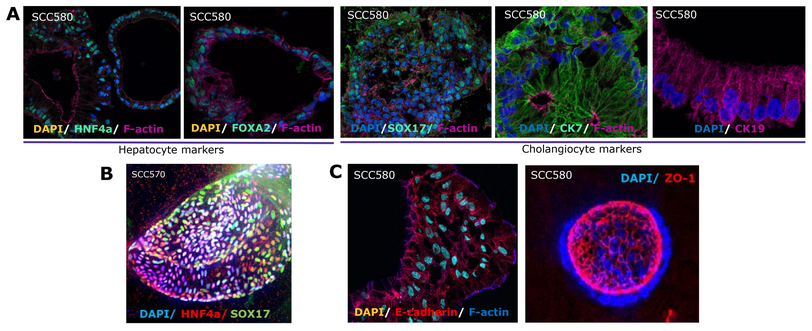

Fig. 6. Representative ICC of liver PDO lines (Cat. No. SCC570 and SCC580). A, Liver PDO express hepatocyte biomarkers HNF4α (MilliporeSigma ZRB1457, clone 4C19), FOXA2 (MilliporeSigma ZRB1872, clone 1H12); and cholangiocyte biomarkers SOX17, CK7 (MilliporeSigma ZRB1769), and CK19. B, Liver PDO are bipotential for hepatocytes and cholangiocytes. C, Liver PDO are polarized and express the basolateral marker E-cadherin (MilliporeSigma 07-697) and apical marker ZO-1. Nuclei in all figures is stained with DAPI. 20x magnification confocal images.

Successful generation of Mature Liver PDO

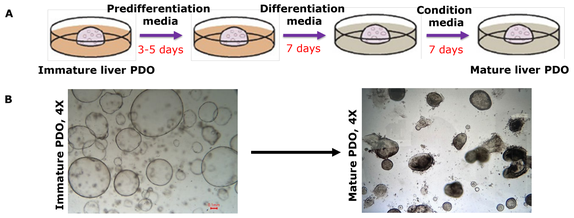

Fig. 7. Robust process to generate mature liver PDO (MLO) from immature liver PDO (ILO). A, Overview of liver PDO maturation protocol; B, Comparative phenotype of ILO and MLO (representative line Cat. No. SCC570). Scale bars, 100 µm.

Not intended for marketing purposes

Mature liver PDO show increased albumin and urea levels

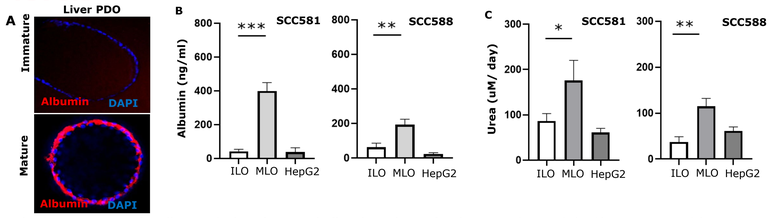

Fig. 8. Upon maturation, liver PDO show increased albumin and urea levels. Mature liver PDO (MLO) were generated from immature liver PDO (ILO) following our in-house SOP. A, ICC with representative MLO line (Cat. No. SCC570) shows higher level of albumin compared to the respective ILO. Albumin and urea levels were also measured using MilliporeSigma kits RAB0603 and MAK471, respectively. MLO lines show higher levels of A, albumin and B, urea, compared to ILO lines, and such increase is donor-dependent. Data shown from representative liver PDO lines, catalog no. denoted in figure.

Mature liver PDO show inducible CYP450 activity

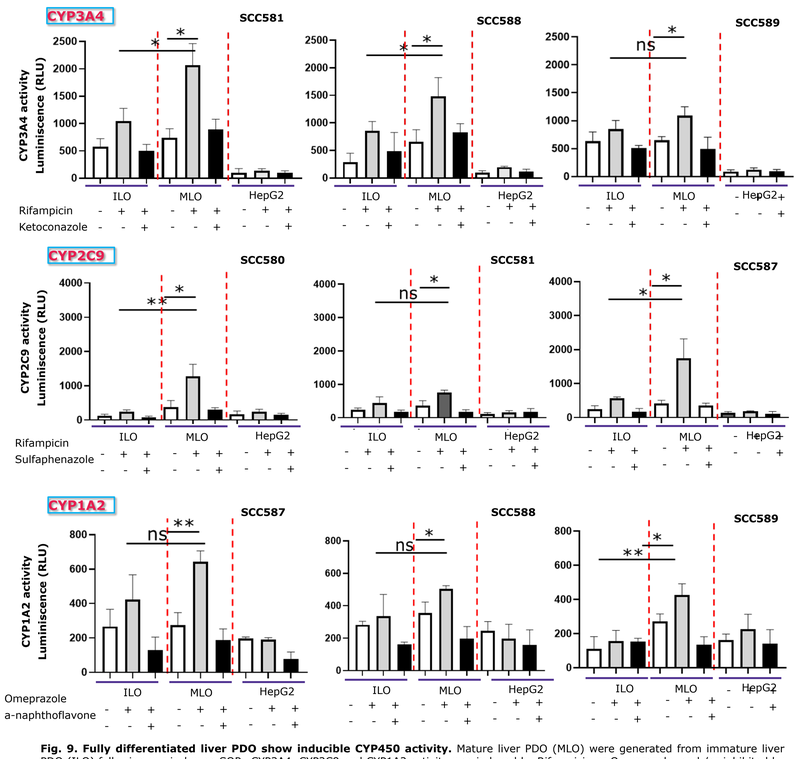

Fig. 9. Fully differentiated liver PDO show inducible CYP450 activity. Mature liver PDO (MLO) were generated from immature liver PDO (ILO) following our in-house SOP. CYP3A4, CYP2C9 and CYP1A2 activity was induced by Rifampicin or Omeprazole, and /or inhibited by Ketoconazole, Sulfaphenazole or a-naphthoflavone, as needed. Luminescence data shows MLO demonstrating higher levels of inducible CYP3A4, CYP2C9 and CYP1A2 activity compared to ILO lines. Increase in CYP450 levels reflects donor-dependent heterogeneity. Data shown from representative liver PDO lines, catalog numbers are denoted in each figure.

Summary

- ❑ We have developed a multi-donor, healthy liver PDO biobank from both fresh and cryopreserved tissue
- ❑ The immature PDO lines are expandable, can be cryopreserved and show robust recovery post-thaw
- ❑ PDO lines express both hepatocyte and cholangiocyte phenotypes, express correct polarity and may be bipotential
- ❑ We have developed robust protocol to generate mature PDO (MLO) from immature PDO (ILO) lines
- ❑ MLO lines show increased albumin secretion, urea production and higher levels of CYP450, and capture donor-dependent heterogeneity
- ❑ Future efforts would include expansion of the liver biobank and development of liver specific assays and developing PDO from liver diseases, including hepatocellular carcinoma, MASLD and NASH.
- ❑ Human organoid models are increasingly recognized as **New Approach Methodologies (NAMs)**. The FDA encourages integration of NAMS to improve translational predictivity and reduce reliance on animal models in preclinical testing.

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HUB ORGANOIDS

MilliporeSigma is the U.S. and Canada Life Science business of Merck KGaA, Darmstadt, Germany.

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