Human organoid-on-a-chip model for therapeutic development in ARPKD

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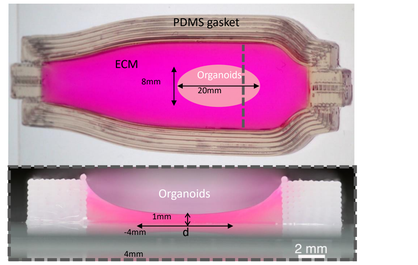
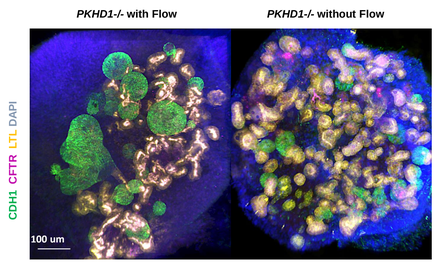
Introduction

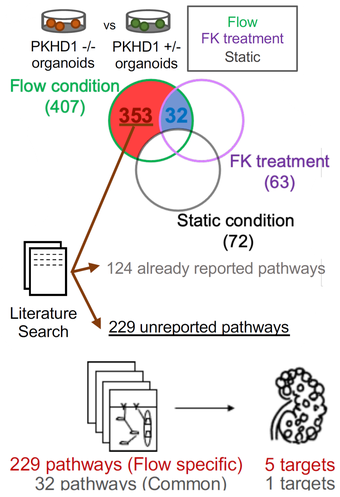
Advances in stem cell biology have enabled the generation of organoids that recapitulate organ-specific 3D architecture, permitting disease modeling in human tissue *in vitro*. However, challenges remain in replicating the physiology of native organs, particularly for diseases whose pathogenesis depend on external stimuli from cellular microenvironments. Autosomal recessive polycystic kidney disease (ARPKD) is a congenital ciliopathy due to mutations in the PKHD1 gene. ARPKD carries high neonatal mortality due to hepatorenal dysfunction and lacks FDA-approved treatments. The paucity of treatments may be due to limitations of ARPKD models in replicating rapidly progressive distal nephron cysts, as Pkhd1$^{-/-}$ mice develop few proximal tubular cysts and PCK rats develop slow proximal and distal nephron cysts. Combining human ARPKD organoids with organ-on-a-chip technology may provide a dynamic cellular microenvironment that simulates disease-specific phenotypes, overcoming limitations of static models to uncover previously unidentified therapeutic targets and compounds for clinical trials.

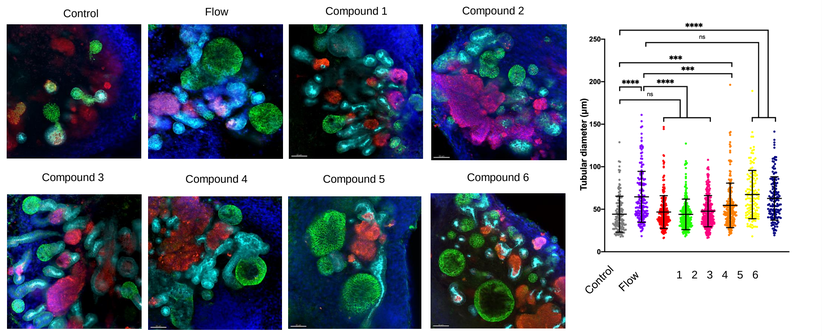
Methods

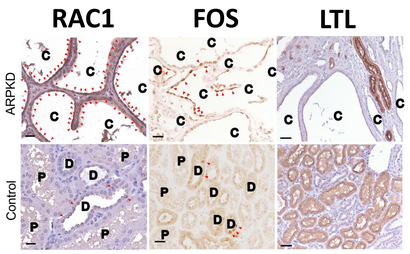
Kidney organoids were made from H9 ES cells, H9-derived *PKHD1* CRISPR mutants, and ARPKD iPSCs. Day 14 organoids were placed onto a 3D-printed millifluidic chip and cultured under flow. Transcriptomics were evaluated by 3D microarray. siRNA targeting siKIF3A was transfected to knock-down cilia. Fluorescent lipid tension reporter (FliptR) was used for FLIM imaging. Kidney sections from four ARPKD patients were assessed by immunohistochemistry.

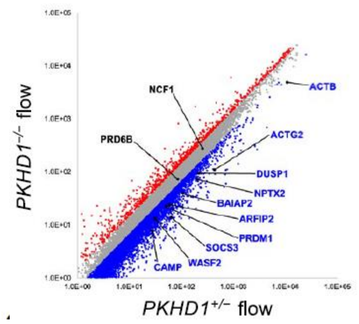


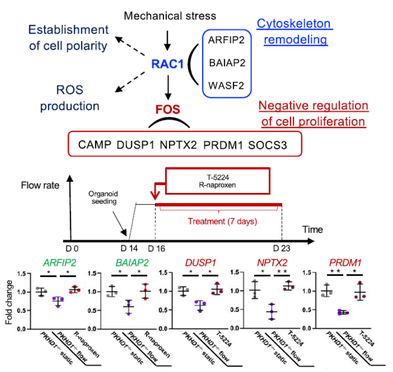












Results

PKHD1$^{-/-}$ organoids-on-chip developed physiologic distal cysts in response to fluid flow, unlike *PKHD1*$^{+/-}$. Microarray demonstrated maturation under flow with 168 GO terms of mechanosensing such as membrane tension, actin cytoskeleton, and cell adhesion. Organoids with knocked-down primary cilia had reduced tubular diameter and proliferation. Tubular membranes stained with FliptR demonstrated longer lifetimes under flow, signifying higher membrane tension. Time-lapse live-imaging of organoids perfused with fluorescent low molecular weight dextran displayed sequential signals in glomeruli then tubular lumens. Comparative gene expression between *PKHD1*$^{-/-}$ and *PKHD1*$^{+/-}$ organoids in response to flow, forskolin, and under static culture permitted the identification of 229 flow-specific signals, and targeted compound screening demonstrated therapeutic effects of RAC1 or FOS inhibition by clinically tested drugs. ARPKD patient samples and *PKHD1*$^{-/-}$ organoids confirmed RAC1 and FOS positivity in cystic epithelia, with FOS demonstrating specificity for distal nephrons.

Summary and Conclusions

Modeling ARPKD with kidney organoids-on-chip demonstrates that flow recapitulates the segment-specificity of cystogenesis seen in afflicted patients. Cyst formation was ameliorated with RAC or FOS inhibition, that might be targeted for therapeutic development.