Title: Organoid Medicine: Modeling ARPKD to discover novel therapeutic targets

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Introduction: Various models in 3D culture have been developed to study polycystic kidney disease (PKD) cystogenesis. A recent study demonstrated the utility of human pluripotent stem cell (hPSC)-derived kidney organoids for modeling autosomal recessive PKD (ARPKD); however, cysts were developed in proximal tubules, which was inconsistent with patient phenotypes. We postulate that vascularized kidney organoids developed in vitro under flow may enable studies of flow-induced mechanosensing signals that might be critical for cystogenesis in humans.

Methods: PKHD1-mutant hPSCs were generated by Clustered regularly interspaced short palindromic repeat (CRISPR)- CRISPR-associated protein 9 (Cas9). Hetero and homozygous mutants with frameshift mutations confirmed by deep-seq were expanded for this study. Kidney organoids were generated following our reported protocol and cultured in vitro under static or flow conditions. Forskolin was also tested for cyst formation. Control and cystic organoids were characterized by immunostaining and transcriptome analyses. To evaluate differential gene expression profiles, 3D-Gene® and Metacore™ were used.

Results: PKHD1-/- organoids formed cysts in both proximal and distal nephrons when treated with forskolin. Forskolin significantly increased cell proliferation marked by KI67 in both tubular cells and interstitial cells. In contrast, fluidic culture enhanced cyst formation in distal nephrons but not in proximal tubules, whereas PKHD1+/- organoids did not form cysts. Further, microarray analysis revealed that 168 GO terms reflected biological processes that are implicated in mechanosensing signals.

Conclusions: The flow-induced ARPKD model demonstrates clinically relevant phenotypes of ARPKD, and transcriptomic analyses suggest the involvement of mechanosensing signals in ARPKD cystogenesis. This novel model complements current PKD models to better understand pathomechanisms for therapeutic development, representing the potential of Organoid Medicine.

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