High-throughput screening in human kidney organoids reveals a role for myosin in ADPKD cystogenesis

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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD), commonly caused by defects in polycystin-1 or polycystin-2, results in the formation of fluid-filled cysts and progressive loss of kidney function. We previously established a cellular model for ADPKD using kidney organoids derived from gene-edited human pluripotent stem cells (hPSC) and uncovered a critical role for microenvironment and adhesion at the early stages of cystogenesis. The molecular pathway underlying ADPKD cystogenesis in this system is not yet fully understood. The goals of the present study are to develop an automated, high-throughput screening (HTS) platform for kidney organoids and to identify modulators of ADPKD cystogenesis, to provide insight into mechanism.

METHODS

To generate organoids compatible with HTS, hPSC were plated in 96-well and 384-well formats and differentiated into kidney lineage for three weeks. Plates were prepared either manually, using multi-channel pipettes, or automatically, using liquid handling robots to perform all steps of plating, differentiation, fixation, and phenotyping. ADPKD organoids were treated with eight candidate factors that might modulate interaction of cells with their surrounding microenvironment, including extracellular matrix components and drugs that modulate adhesion.

RESULTS

Automated differentiation in microplate formats resulted in the formation of numerous kidney organoids per well containing proximal tubule, distal tubule and podocyte cells in distal-to- proximal arrangements. Gene-edited ADPKD kidney organoids produced cysts from kidney tubules, similarly to large format wells. While none of the treatment conditions tested resulted in a dose-dependent decrease in cystogenesis, blebbistatin, a specific inhibitor of non-muscle myosin II (NMII), induced a significant increase in cyst formation. Further validation in low- throughput suspension cultures confirmed that blebbistatin significantly increased both the diameter and number of cysts in ADPKD organoids, compared to isogenic controls.

CONCLUSIONS

We have established a platform for disease modeling and phenotypic screening of mini-kidney organoids in HTS formats with potential applications for predicting safety and efficacy of drugs for ADPKD and other diseases. Applying our HTS platform, we identified blebbistatin, an inhibitor of NMII, as a specific activator of ADPKD cystogenesis. This finding suggests that the polycystins may positively regulate actomyosin activation within the tubular epithelium, strengthening and tightening the tubule and preventing it from deforming into a cyst.