Title:

A novel organoid model for autosomal dominant polycystic kidney disease based on mouse nephron progenitor cells (NPCs)

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Introduction:

An *in vitro* cell culture system that accurately recapitulates ADPKD cystogenesis is needed, and a human iPS or ES cell-derived organoid system is one of the options. However, the process takes >20 days from iPS or ES cells to organoid with cystic structures and differentiation efficiency varies between cell lines. In addition, long-term methods of induction of differentiation result in higher inter-experiment variability. Moreover, the the results of human-based *in vitro* studies cannot be easily assessed in a whole organism. To overcome these problems, we have established a mouse nephron organoid system from primary nephron progenitor cells (NPCs).

Methods:

Antibodies that detect kidney lineage differentiation markers were used to sort NPCs from mouse E13.5 embryonic kidneys. Sorted NPCs were maintained in 3D expansion culture and evaluated for their nephrogenic potential. We modified conditions to assess organoid differentiation using both air-fluid and suspension culture methods. Finally, we used established differentiation protocols to evaluate cystogenesis in nephron organoids derived from NPCs of ADPKD mouse models.

Results:

Robo2^{high}/Pdgfrb^{-}/Podocalyxin^{-} cells (NPCs) sorted from E13.5 embryonic WT and ADPKD models kidneys could be cultured for >40 passages and maintain nephrogenic potential, and they could be differentiated into nephron organoids using either air-fluid or suspension culture methods in <10 days. NPCs established from ADPKD models spontaneously formed cystic structures without inducers like forskolin in suspension culture but not in air-fluid culture.

Conclusions:

We have established a novel mouse nephron organoid system based on an antibody-based flow-sorting system. NPCs sorted with our protocol could be maintained long term with nephrogenic potential.

ADPKD mutants spontaneously make cystic structures when grown in suspension. This is the first mouse NPC-based organoid system that appears to make cystic structures spontaneously and will be a useful tool to reveal mechanisms of cystogenesis in ADPKD.

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