3D Organoids Reveal a Critical Role for Microenvironment in Polycystic Kidney Disease

Benjamin "Beno" Freedman Corning 3D summit October <u>17</u>, 2018

Organoids mimic organ patterning





or·ga·noid (n.) - a multi-cellular unit *in vitro* that resembles a tissue or organ of the body (structurally and functionally)

Growing new kidney tissue from stem cells



Disease modeling

Freedman et al., *Nat. Comm.* (2015) Freedman, *Biomarker Insights* (2015)







Growing kidney organoids from iPS cells

Using CRISPR to re-create human disease



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Differentiation of iPS cells into NPC and organoids



Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids



Organoids resemble primitive kidneys



Taguchi et al., *Cell Stem Cell*, 2014 Takasato et al., *Nature*, 2015 Freedman et al., *Nat. Commun.*, 2015 Morizane et al., *Nat. Biotech*, 2015

Kidney organoids contain nephron-like structures



https://www.studyblue.com/notes/n ote/n/nephron-slide/deck/3096333

Kim et al., Stem Cells (2017)

High throughput differentiation of iPS-derived organoids



Automated analysis of drug treatments







CHIR99021 (µM)





podocyte area 📃 distal tubule area

Czerniecki et al., Cell Stem Cell (2018)







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Polycystic kidney disease



- epithelial cysts and fibrosis cause declining kidney function
- dominant mutation in *PKD1* or *PKD2* (1 in 600 adults)
- liver, other organs also affected
- better lab models needed for human pathogenesis

The cellular pathophysiology of PKD is unclear



Torres and Harris, Annu Rev. Med, 2010 Torres & Harris, Kidney Int., 2009

Generation of *PKD1^{-/-}* and *PKD2^{-/-}* stem cells



- A1 TGTGCCCCGGTACGG CCACCC
- A2 TGTGCCCCGCCGTACGG CCAC



- WT GCCGCGATAACCCCGG CTTCG
- A1 GCCGCGATAACCCCGG CTTCA
- A2 GCCGCGATACCCCGG CTTCGA





Freedman et al., *Nat. Comm.* (2015) Cruz et al., *Nat. Mat.* (2017)

PKD1^{-/-} and **PKD2**^{-/-} organoids form cysts



Historically, PKD-specific cyst formation has been very difficult to re-create in vitro.

Visualizing PKD-specific cyst formation



Cruz et al., Nat. Mat. (2017)

Testing the role of microenvironment in cystogenesis



Cysts phenotypically resemble human PKD









Model of organoid PKD cystogenesis





LETTERS PUBLISHED ONLINE: 2 OCTOBER 2017 | DOI: 10.1038/NMAT4994 nature materials

Organoid cystogenesis reveals a critical role of microenvironment in human polycystic kidney disease

suspension culture

Screening reveals a new pathway in PKD



Toxicity assessment in HTS organoid plates







Czerniecki et al., Cell Stem Cell (2018)







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Injections into neonatal kidney cortex





Freedman et al., Nat Comms. 2015

proximal tubule/human/DNA



mCherry NPCs



Li et al., Cell Stem Cell, 2016



This is harder to do in adult kidneys.

Conclusions



Human pluripotent stem cells can produce kidney organoids with complex, nephron-like structures.

Gene-edited organoids and 3D culture reveal a critical role for microenvironment in human PKD.

High-throughput systems incorporating organoids can be used to probe disease pathways and profile drugs

Organoids can survive after transplantation and may in the future provide a new source of autologous graft



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National Kidney

PKD FOUNDATION

Polycystic Kidney Disease

Foundation"

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