Using chILD patient-derived induced pluripotent stem cells to model ABCA3 dysfunction in vitro

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Introduction

Childhood interstitial lung disease (chILD) can be caused by autosomal recessive mutations in ATP binding cassette member A3 (ABCA3), a lamellar body associated lipid transporter expressed in alveolar epithelial type II cells (AEC2s). Dysfunction of ABCA3 is thought to cause AEC2 injury by disrupting surfactant biogenesis, resulting in lung remodeling. AEC2s are difficult to study in cell culture due to their propensity to transdifferentiate, and inability to adequately proliferate. Using patient-specific induced pluripotent stem cells (iPSCs) as an inexhaustible source of AEC2s, we sought to engineer an *in vitro* model of ABCA3 deficiency.

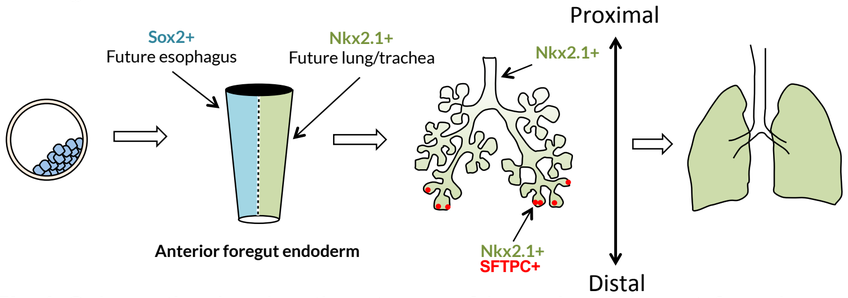

Fig.1 Schematic showing the stages of lung development from inner cell mass to ventral anterior endoderm with Nkx2.1 expression, a marker found in all developing lung epithelia. SFTPC is the first locus specifically activated during differentiation of distal lung epithelial progenitors.

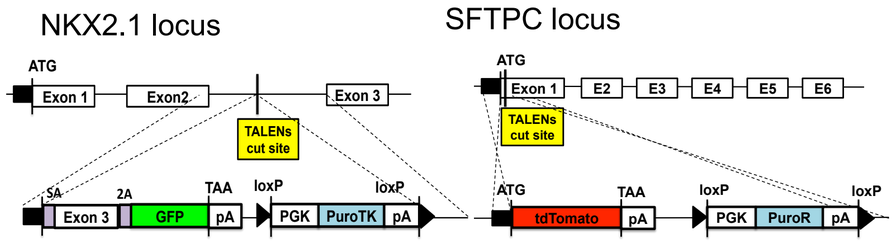

Fig.2 Using gene editing technology, we targeted a GFP fluorochrome reporter to the NKX2.1 locus and a Tomato fluorochrome reporter to the Surfactant protein C (SFTPC) gene, the first locus specifically activated during the differentiation of distal lung epithelial progenitors.

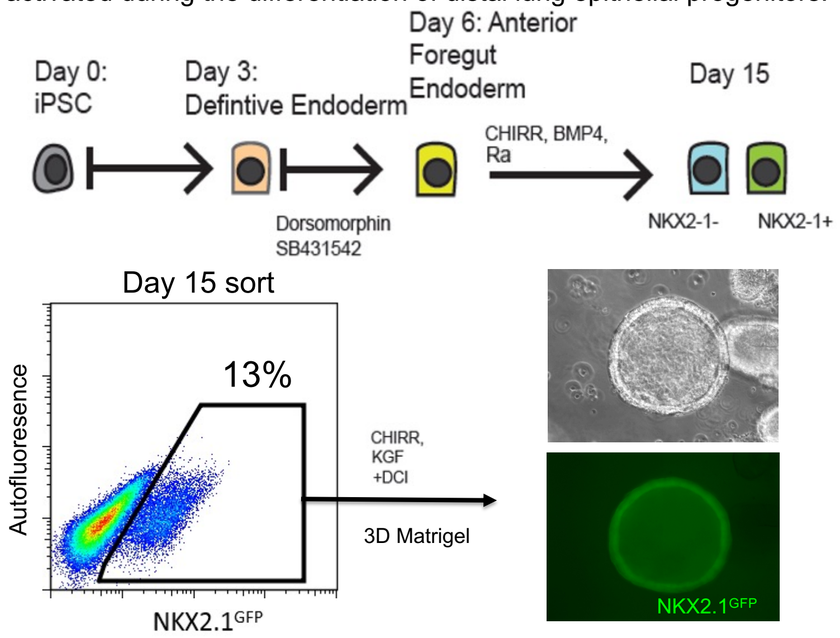

Fig.3 Using the reporters to identify, track, and purify iPSC-derived cells undergoing lung differentiation, we established a protocol which mimics the natural *in-vivo* stages of embryonic lung development.

Directed Differentiation of Human Pluripotent Stem Cells to Lung epithelial Progenitors

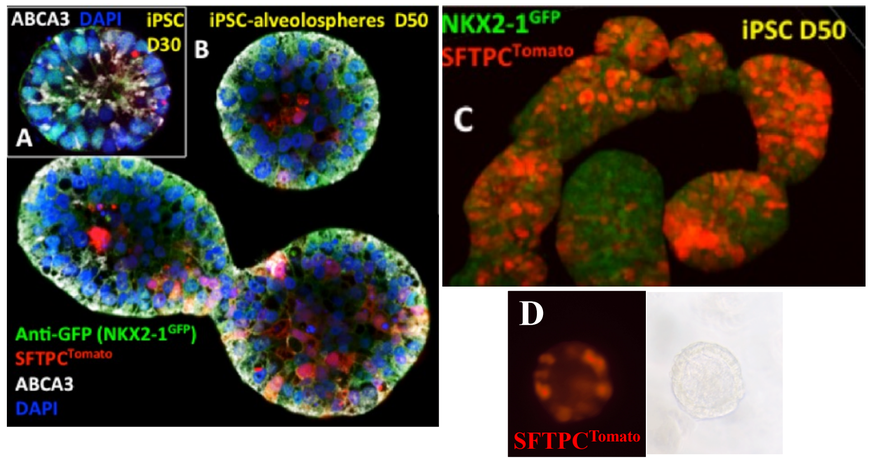

Fig.4 iPSC-derived "alveolospheres" with AT-2 like cells: A) iPS17 cell line sorted on day 16 for NKX2-1^{GFP} immunostained for ABCA3. B) Day 50 culture of the same cells showed co-expression of ABCA3 and NKX2-1^{GFP}. C) 3D confocal reconstruction of cells from B showed both NKX2-1 and SFTPC positive AT-2 like cells. D) AT2 proliferative potential demonstrated by sorting SFTPC$^{tomato+}$ /NKX2-1^{GFP+} cells from alveolospheres on day 36 shows clonal proliferation on day 45

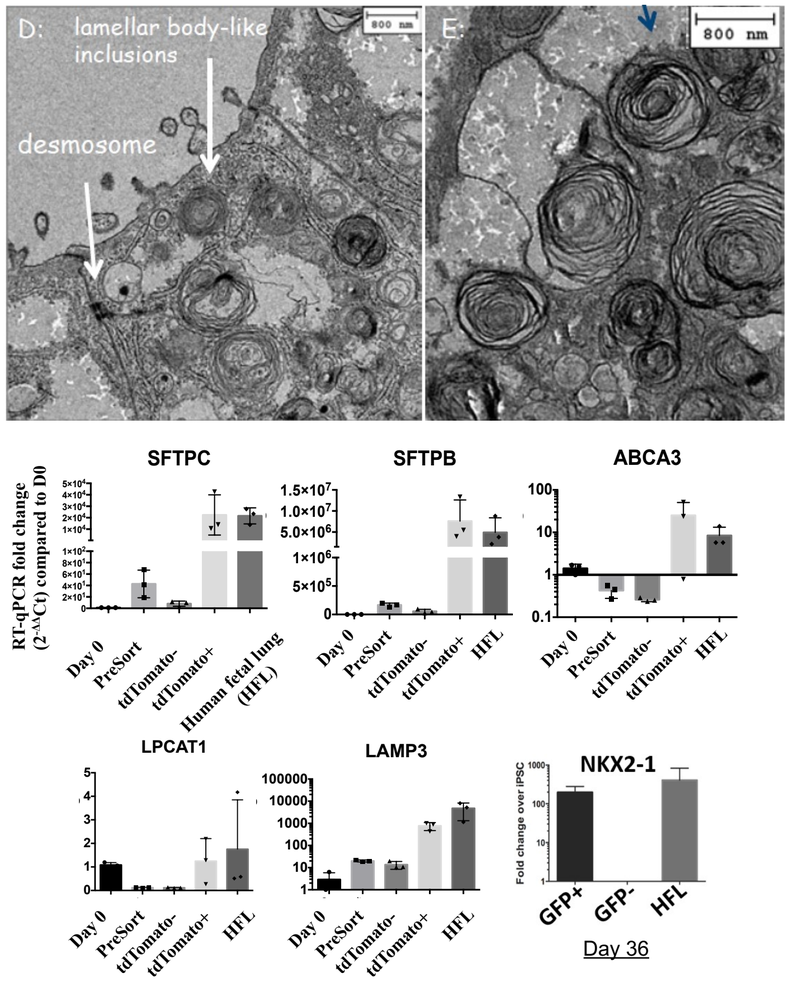

Fig.5 Gene expression to human fetal lung (HFL) control using RT-qPCR showed similar levels of SFTPC and transcripts encoding lamellar body associated proteins, including-- ABCA3, LAMP3, LPCAT, and SFTPB on Day 30. Similar level of NKX2-1 levels was also observed on Day 36 analysis. Furthermore, ultrastructural analysis using TEM revealed lamellar body-like inclusions.

ABCA3 Disease Modeling Using iPSC-derived AT2

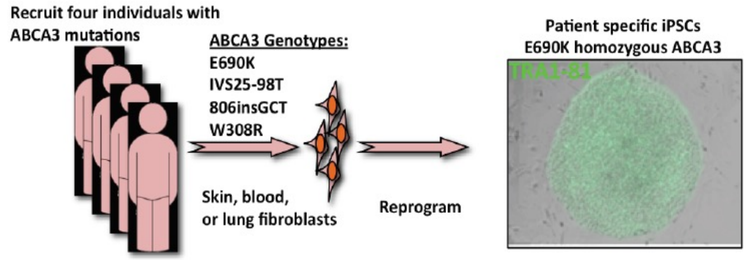

Fig.6 Schematic showing the work flow of generating 4 different homozygous ABCA3 mutant lines. Patient cell samples were taken either from blood PBMC or dermal fibroblasts. We have already reprogrammed a blood sample from a patient with lung disease from a homozygous E690K ABCA3 mutation.

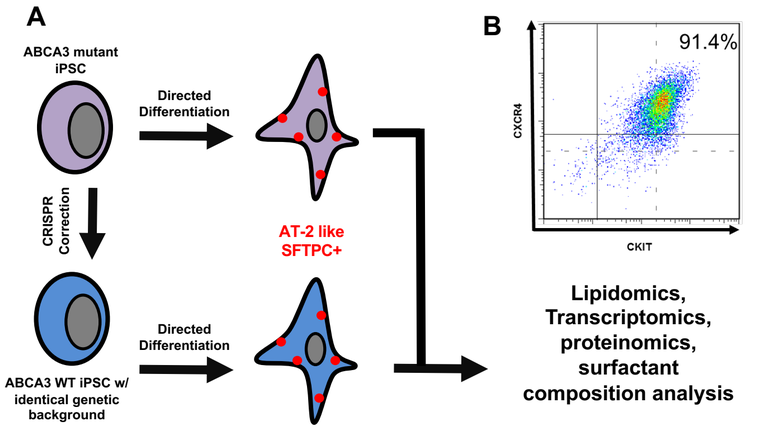

Fig.7 A) Each ABCA3 mutant line will be genetically engineered to have a SFTPC^{Tomato} reporter as depicted in Fig 2 to observe the extent of differentiation comparable to such shown in fig 4,5. Furthermore, ABCA3 mutant lines will be CRISPR corrected to generate wildtype lines with similar genetic background, allowing analysis on transcriptomic, lipidomic, and proteomic analysis of each type of homozygous ABCA3 mutation B) Day 4 differentiation of E690K ABCA3 homozygous mutant line demonstrated positivity for both CKIT and CXCR4, both of which are definitive endoderm markers.

Conclusion

We demonstrated the ability to generate putative AECs of sufficient maturity to potentially model chILD caused by mutations in ABCA3. Using this protocol, we will generate AEC2 models of different ABCA3 mutations compared to their gene-corrected controls. Upon complete characterization, these models will allow robust testing of novel drugs and gene editing techniques for potential use in patients with ABCA3 mutations.

References/Acknowledgements

Besnard, V. *et al.* Conditional deletion of Abca3 in alveolar type II cells alters surfactant homeostasis in newborn and adult mice. *Am J Physiol Lung Cell Mol Physiol* **298**, L646 (2010).
Kurmann, A.A. *et al.* Regeneration of Thyroid Function by Transplantation of Differentiated Pluripotent Stem Cells. *Cell Stem Cell* **17**, 527 (2015).
Longmire, T.A. *et al.* Efficient derivation of purified lung and thyroid progenitors from embryonic stem cells. *Cell Stem Cell* **10**, 398 (2012).
Cardoso, W.V. & Kotton, D.N. Specification and patterning of the respiratory system, in *StemBook* (Cambridge (MA); 2008).
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