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.

ABCA3 Disease Modeling Using Human Pluripotent Stem Cells

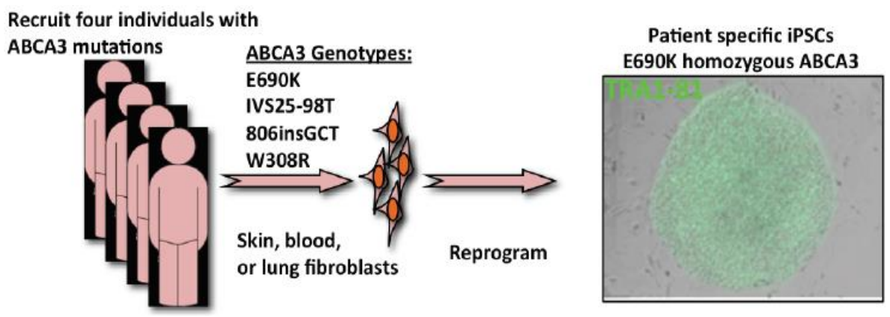

Fig.1 Schematic showing the work flow of generating 4 different homozygous ABCA3 mutant lines. Patient cell samples were taken either from blood PBMC or dermal fibroblasts. We have already reprogrammed two iPSC lines from a patient with lung disease from a homozygous E690K and W308R ABCA3 mutation.

ABCA3 Disease Modeling Using A549

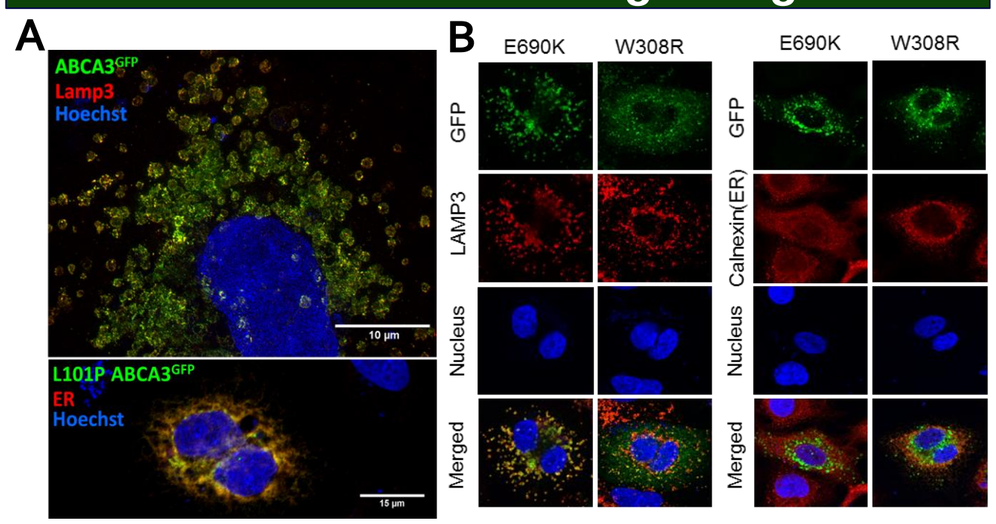

Fig.2 (A) Immunofluorescence of A549 lung adenocarcinoma cell transiently expressing wildtype or L101P mutant ABCA3^{GFP} (B) A549 transfected with plasmid expressing E690K and W308R mutant ABCA3-GFP fusion proteins stained against ER, LAMP3.

Directed Differentiation of Human Pluripotent Stem Cells to Lung Epithelial Progenitors

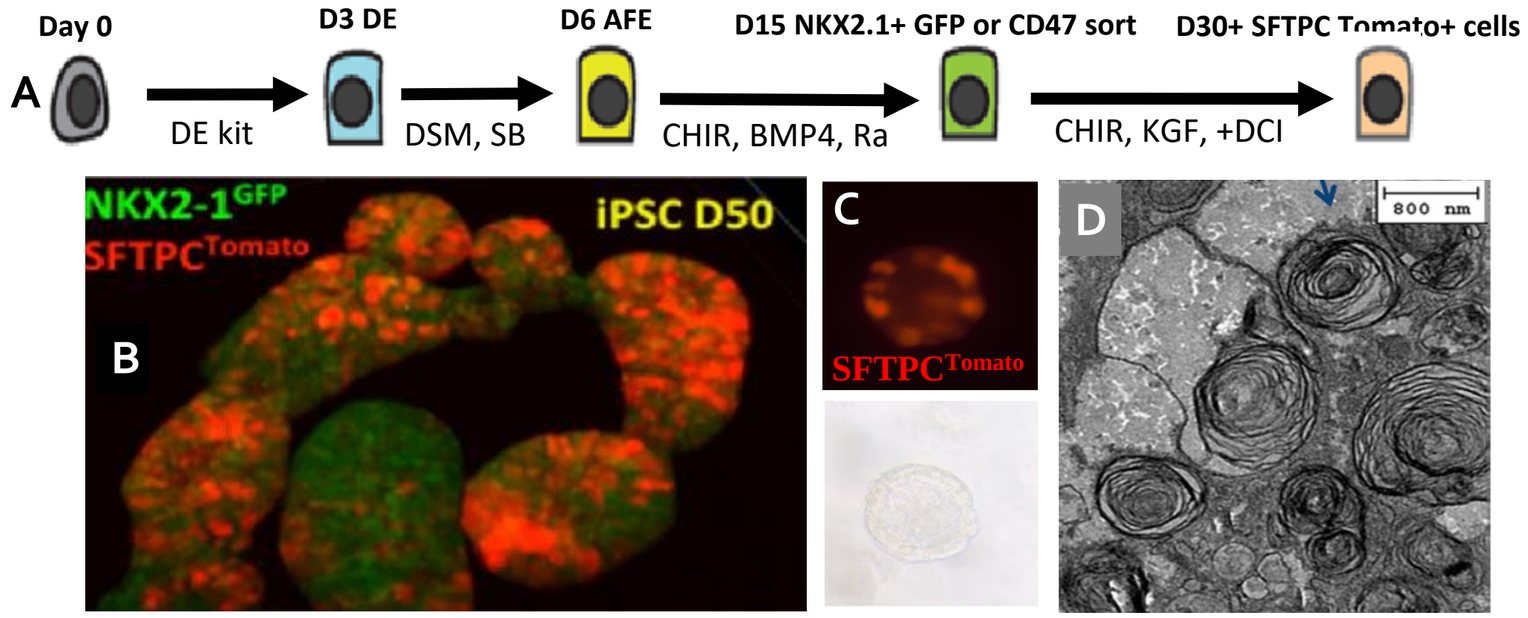

Fig.3 iPSC-derived "alveolospheres" with AT-2 like cells: A) Directed differentiation of iPSC to definitive endoderm (DE), anterior foregue endoderm (AFE) and SFTPC+ lung epithelial progenitors B) 3D confocal reconstruction showed both NKX2-1 and SFTPC positive AT-2 like cells. C) AT2 proliferative potential demonstrated by sorting SFTPC$^{tomato+}$/NKX2-1^{GFP+} cells on day 36 shows clonal proliferation on day 45. D) Ultrastructural analysis using TEM revealed lamellar body-like inclusions in iPSC-derived AT2s

ABCA3 mutant iPSC-derived Lung Epithelial Progenitors

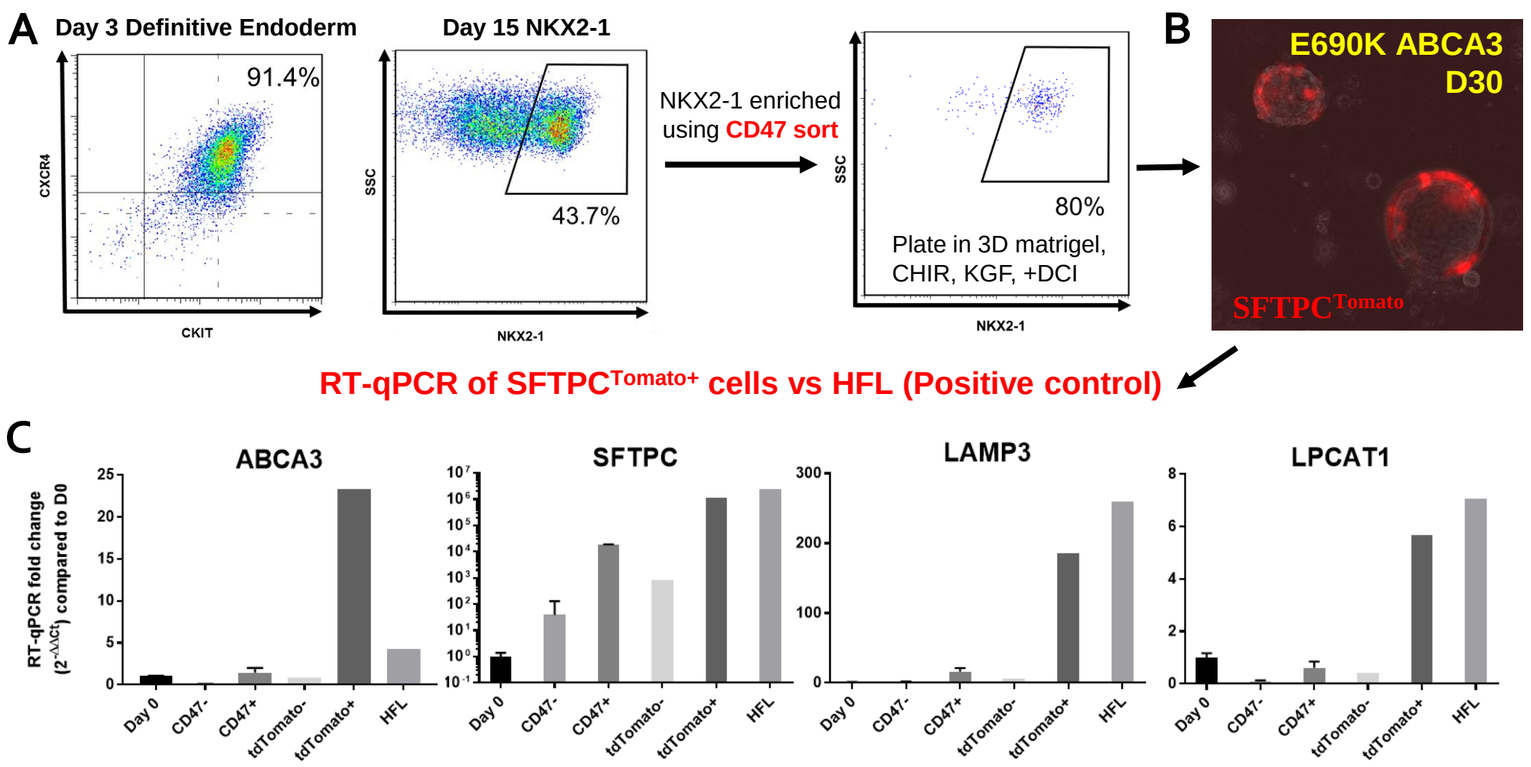

Fig.4 Derivation of AT2-like cells containing E690K ABCA3 mutation (A) FACS plots showing derivation of DE, D15 NKX2-1 expression, post CD47 sort enrichment of NKX2-1, and D30 SFTPC$^{Tomato+}$ cell population. (B) Fluorescent microscopy showing the same D30 cells expressing SFTPC^{Tomato}. (C) Gene expression comparing D30 Tomato+ cells to week 21 human fetal lung (HFL) positive control using RT-qPCR showed similar or greater levels of SFTPC, ABCA3, LAMP3 and LPCAT1.

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