Modeling Cystic Fibrosis Using Pluripotent Stem Cell-Derived Human Pancreatic Ductal Epithelial Cells

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ABSTRACT
We established an efficient strategy to direct human pluripotent stem cells, including human embryonic stem cells (hESCs) and an induced pluripotent stem cell (iPSC) line derived from patients with cystic fibrosis, to differentiate into pancreatic ductal epithelial cells (PDECs). After purification, more than 98% of hESC-derived PDECs expressed functional cystic fibrosis transmembrane conductance regulator (CFTR) protein. In addition, iPSC lines were derived from a patient with CF carrying compound frameshift and mRNA splicing mutations and were differentiated to PDECs. PDECs derived from Weill Cornell cystic fibrosis (WCCF)-iPSCs showed defective expression of mature CFTR protein and impaired chloride ion channel activity, recapitulating functional defects of patients with CF at the cellular level. These studies provide a new methodology to derive pure PDECs expressing CFTR and establish a “disease in a dish” platform to identify drug candidates to rescue the pancreatic defects of patients with CF.

SIGNIFICANCE
An efficient strategy was established to direct human pluripotent stem cells, including human embryonic stem cells (hESCs) and an induced pluripotent stem cell line derived from patients with cystic fibrosis (CF-iPSCs), to differentiate into pancreatic ductal epithelial cells (PDECs). After purification, more than 98% of hESC-PDECs derived from CF-iPSCs showed defective expression of mature cystic fibrosis transmembrane conductance regulator (CFTR) protein and impaired chloride ion channel activity, recapitulating functional pancreatic defects of patients with CF at the cellular level. These studies provide a new methodology for deriving pure PDECs expressing CFTR, and they establish a “disease-in-a-dish” platform for identifying drug candidates to rescue the pancreatic defects of these patients.

INTRODUCTION
Cystic fibrosis (CF) is an inherited disease of the secretory glands, seriously affecting multiple organs, including the pancreas, liver, lungs, and intestines. The pancreas is among the earliest and most seriously affected organs impacted by CF. Several animal models have been established to study CF; however, the severity of CF pancreatic pathology at birth differs widely across species, being most severe in pigs [1], similar in ferrets and humans [2], and least severe in mice [3]. More than 1,000 genomic mutations have been identified in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. There is, therefore, a clear need to develop a human model to study the pancreatic insufficiency of patients with CF who carry different genomic mutations.

Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (iPSCs) provide systems to study the developmental dysfunction in genetic disease, as well as a platform to recapitulate the cellular pathology of the disease [4]. Several groups have successfully generated lung epithelial cells derived from iPSC lines derived from patients with cystic fibrosis (CF-iPSCs), documenting decreased expression of CFTR protein, and impaired chloride ion channel activity [5–10]. More recently, CF-iPSC-derived cholangiocytes have been used to model biliary dysfunction of patients with CF [8, 11]. However, there has been no human iPSC model to study the pancreatic defects of these patients, mainly because of the lack of an efficient differentiation strategy to make functional pancreatic duct epithelial cells (PDECs).

Here, we present an efficient protocol to differentiate, in a stepwise manner, human pluripotent stem cells to PDECs. In addition, functional PDECs were derived from CF-iPSCs (CF-PDECs),...
which displayed defective expression of mature CFTR protein and impaired Cl⁻ channel activity.

**MATERIALS AND METHODS**

**Cell Culture**

hESC and iPSC lines were routinely cultured with irradiated mouse embryonic fibroblast (MEF) feeders on tissue culture plates precoated with 0.1% gelatin in water. The control and CF fibroblasts were obtained from the Coriell Cell Repository (Coriell Institute, Camden, NJ, https://catalog.coriell.org). Human pancreatic ductal epithelial cells were purchased from American Type Culture Collection (CRL-4036; Manassas, VA, http://www.atcc.org). Additional details of procedures used in this study are presented in the supplemental online data.

**PDEC Differentiation**

hPSCs cultured with irradiated MEF feeders on tissue culture plates precoated with 0.1% gelatin in water were treated with 3 μM CHIR99021 and 100 ng/ml Activin A in Roswell Park Memorial Institute (RPMI) medium for 1 day, and then 100 ng/ml Activin A in RPMI supplemented with 0.2% fetal bovine serum (FBS). Then the medium was changed to 50 ng/ml FGF7 in RPMI supplemented with 2% FBS and maintained for 2 days. Cells were cultured with 300 nM LDN193189, 2 μM retinoic acid, and 0.25 μM SANT-1 in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 1:3 B27; fetal bovine serum; hESC, human embryonic stem cell; n.s., no significant difference; PDEC, pancreatic ductal epithelial cell; PP, pancreatic progenitor; RPMI, Roswell Park Memorial Institute medium; SCC, side scatter; VPA, valproic acid.

**Figure 1.** Directed differentiation from hESCs to PDECs. (A): Scheme of the directed differentiation protocol. (B): Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of HUES8-derived PDECs that were treated with 300 nM LDN193189, 10 ng/ml BMP2, 10 ng/ml BMP4, or 10 ng/ml BMP2 plus 10 ng/ml BMP4 during differentiation (n = 3 independent experiments). (C): Intracellular flow cytometry analysis of HUES8-derived PDECs that were treated with 10 ng/ml BMP4 from D16 to D27 (n = 3 independent experiments). (D): Intracellular flow cytometry analysis of HUES8-derived PDECs that were treated with 10 ng/ml BMP4 plus 20 μM VPA from D16-D27 (n = 3 independent experiments). (E): qRT-PCR analysis of PDEC marker genes, including CFTR, CK19, CAII, and BEST1, in HUES8 cells, DE, PP, and PDECs (n = 3 independent experiments). (F): Intracellular flow cytometry analysis of HUES8-derived PDECs using CK19, CFTR, and SOX9 antibodies. Scale bars = 10 μM. Data are presented as mean ± SD; p values were calculated by unpaired two-tailed Student t test. *, p < .05; **, p < .01. Abbreviations: BMP, bone morphogenetic protein; CFTR, cystic fibrosis transmembrane conductance regulator; D, day; DE, definitive endoderm; DMEM, Dulbecco’s modified Eagle’s medium; DMEM+B27, Dulbecco’s modified Eagle’s medium supplemented with 1:3 B27; FBS, fetal bovine serum; hESC, human embryonic stem cell; n.s., no significant difference; PDEC, pancreatic ductal epithelial cell; PP, pancreatic progenitor; RPMI, Roswell Park Memorial Institute medium; SCC, side scatter; VPA, valproic acid.
Construction of the Lentiviral Construct Containing CFTR-Puro-EF1α-ClopHensor

The CFTR channel and pH sensor contained in the plasmid pcDNA3-ClopHensor (Addgene, Cambridge, MA, https://www.addgene.org) were amplified by polymerase chain reaction (PCR) producing a 2.455-kb product. The following product was digested with Clal and XhoI. The pVLX-mCherry-C1 vector (Clontech Laboratories, Mountain View, CA, https://www.clontech.com) was similarly digested. The two products were then ligated and transformed in Dam−/Dcm− competent Escherichia coli (New England Biolabs, Ipswich, MA, https://www.neb.com). The minimum CFTR promoter (372 bp) was inserted into the newly created plasmid by digestion of both the plasmid and the PCR product with EcoRI and AgeI. The two products were then ligated and transformed in DH5α competent E. coli (New England Biolabs), resulting in the lentiviral construct containing CFTR-Puro-EF1α-ClopHensor.

Iodine Efflux Assay

PDECs were dissociated and replated onto 35-mm dishes (ibidi, Martinsried, Germany, http://ibidi.com). Two types of buffer were used, including I−-free buffer (138 mM Na gluconate, 2 mM Ca gluconate, 0.8 mM NaH2PO4, 1 mM Mg gluconate, 5.4 mM K gluconate, and 10 μM forskolin) and iodine loading buffer (138 mM NaI, 2 mM CaCl2, 0.8 mM NaH2PO4, 1 mM MgCl2, 5 mM KI, and 10 μM forskolin). Cells were exposed to control I−-free buffer for 20 minutes as influx control and an additional 20 minutes as efflux control. For iodine efflux assays, cells were cultured with iodine loading buffer for 20 minutes and then cultured in I−-free buffer for 20 minutes. Images were collected by >20 live-cell oil immersion objective lens. Image processing was done with Metamorph software (Molecular Devices, Sunnyvale, CA, http://www.moleculardevices.com).

Statistical Analysis

Independent experiments were performed three times, if not mentioned separately. The p values were calculated by unpaired two-tailed Student t test. Data are reported as mean ± SD, unless otherwise indicated.

RESULTS

We first focused on developing an efficient strategy to differentiate hPSCs to CFTR-expressing PDECs. Previously, we identified synthetic small molecules that promote the generation of definitive endoderm (DE) [12] and pancreatic progenitors (PPs) [13], respectively (Fig. 1A). The spontaneous differentiation of progenitors gives rise to less than 9% PDECs [13] (supplemental online Fig. 1). In addition, these PDECs do not have functional Cl− channel activity (supplemental online Fig. 2). We decided to screen a small library of growth factors and chemicals to test their capacities to induce differentiation from PPs toward functional PDECs (supplemental online Fig. 3).

After screening more than 20 growth factors and chemicals, we found that bone morphogenetic protein (BMP) and valproic acid (VPA) play critical roles in the generation of CFTR+ cells. To determine the role of the BMP signaling pathway during the differentiation, the HUES8-derived PP population was treated with 300 nM LDN193189 (a BMP antagonist), 10 ng/ml BMP2, 10 ng/ml BMP4, or 10 ng/ml BMP2 plus 10 ng/ml BMP4, respectively, and subsequently analyzed with quantitative real-time PCR (qRT-PCR) assays. We found that BMP4 treatment increased CFTR mRNA expression by 2.5-fold (Fig. 1B). BMP2 partially mimics the effect of BMP4, but cannot synergize with BMP4 to further increase CFTR expression. In contrast, LDN193189, a BMP antagonist, decreased the expression of CFTR (Fig. 1B). After 7 days of treatment with 10 ng/ml BMP4, 22.5% ± 2.4% cells were positively stained by CFTR antibody (Fig. 1C; supplemental online Fig. 1a). Together, the data indicate that BMP signaling is both essential and sufficient to induce CFTR expression. Previous studies suggested that histone deacetylase inhibitors, such as VPA, promote acinar and ductal differentiation in mouse development [14]. We found that 20 μM VPA treatment further increased CFTR mRNA expression in the presence of BMP4 (Fig. 1D). Consistently, VPA treatment increased the percentage of CFTP+ cells from 22.5% ± 2.4% to 43.4% ± 7.6%, as indicated by intracellular flow cytometry (Fig. 1E; supplemental online Fig. 1a).

In summary, we established a directed differentiation strategy including 3 days of treatment with 100 ng/ml FGF10, followed by 7 days of treatment with 20 μM VPA plus 10 ng/ml BMP4 (Fig. 1A). After 23 days of differentiation, more than 40% of a HUES8-derived cell population expressed CFTR, indicated by intracellular flow cytometry (Fig. 1E). In addition, qRT-PCR analyses suggested that HUES8-derived PDECs show increased expression of CFTR and other PDEC markers, such as cytokeratin 19 (CK19), carbonic anhydrase II (CAII) [15], and bestrophin (BEST1) [16]. The expression level of these PDECs markers in hESC-derived PDECs was comparable to that of a human PDEC line (Fig. 1F; supplemental online Fig. 4). Moreover, immunocytochemistry...
Figure 3. iPSC derivation and characterization. (A): WCCF-iPSC and WCC-iPSCs show typical embryonic stem cell-like colony morphology and express pluripotency markers. Scale bars = 50 μm. (B): The mutations of WCCF-iPSCs were confirmed using genomic DNA sequencing; WCC- and WCCF-iPSCs are capable of differentiating into PDX1-positive PPs and CK19+/SOX9+ PDECs (n = 3 independent experiments). Scale bars = 10 μm. (E): Quantification of CK19+/SOX9+ cells in WCC- and WCCF-iPSC-derived PDECs (n = 3 independent experiments). (F): Quantitative real-time polymerase chain reaction analysis of PDEC marker genes, including CFTR, CK19, CAII, and BEST1, in WCC-iPSC- and WCCF-iPSC-derived PDECs. Data are presented as mean ± SD; p values were calculated by unpaired two-tailed Student t test. Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; DAPI, 4′,6-diamidino-2-phenylindole; iPSC, induced pluripotent stem cell; n.s., no significant difference; PDEC, pancreatic ductal epithelial cell; PP, pancreatic progenitor; WCC, Weill Cornell control patient; WCCF, Weill Cornell cystic fibrosis patient.
was used to validate the expression of PDEC markers. Consistent with qRT-PCR results (Fig. 1F), no CFTR\(^+\), CK19\(^+\), PDX1\(^-\), or SOX9\(^-\) cells were detected at the DE stage (supplemental online Fig. 5a). Likewise, PPs expressed PDX1 and SOX9 but not CFTR (supplemental online Fig. 5a). Furthermore, HUES8-derived PDECs stained positively with antibodies against PDEC markers, including CK19, SOX9, and CFTR (Fig. 1G). The immunostained cells were quantified using MetaMorph Image Analysis Software (Molecular Devices). More than 95% of differentiated cells expressed SOX9 and more than 90% expressed CK19. More importantly, Western blotting experiments demonstrated the expression of fully mature CFTR protein band C at approximately 160 kDa (Fig. 2A) [17].

To purify the CFTR-expressing PDECs, a lentivirus vector was constructed that contained a CFTR-promoter directing expression of the puromycin-resistance gene (for selected expression of the vector in PDECs) and a cytomegalovirus (CMV) promoter that drives expression of the ClopHensor protein. The CFTR promoter has been validated previously [18]. The ClopHensor was previously designed to monitor in real time the intracellular \(\mathrm{I}^-\) and \(\mathrm{Cl}^-\) concentrations [19]. The CFTR-Puro-EF1\(\alpha\)-ClopHensor vector was used to purify the PDECs and subsequently measure \(\mathrm{Cl}^-\) channel activity. HUES8 cells were differentiated to the PDEC stage, transduced with lentivirus carrying CFTR-Puro-EF1\(\alpha\)-ClopHensor, and selected with puromycin. A lentivirusing EF-1\(\alpha\)-mCherry-Puro was used as a control to monitor the infection efficiency and validate the puromycin selection effect. The infection efficiency is approximately 50%, based on mCherry expression (data not shown). After puromycin selection, the CFTR\(^+\) cells were enriched from approximately 40% (Fig. 1E, before puromycin selection) to more than 98% (Fig. 1F; supplemental online Fig. 1a), as indicated by flow cytometry analysis. Western blot experiments further confirmed the enrichment of CFTR-expressing PDECs after puromycin treatment (Fig. 2A). Consistent with Western blot and flow cytometry results, qRT-PCR experiments indicated that the expression levels of PDEC markers, such as CFTR, CK19, CALI, and BEST1, are three- to fourfold higher in PDECs after puromycin selection than in PDECs before selection (Fig. 2C). Finally, the protocol generated PDECs with functional \(\mathrm{Cl}^-\) channel activity (described below).

To confirm CFTR/\(\mathrm{SOX9}^+\)/CK19\(^+\) cells derived using our current protocol were of pancreatic origin, HUES8-derived PP and PDEC populations were stained with antibodies against \(\alpha\)-fetoprotein (a marker of liver and bile duct precursors), albumin (a marker of hepatocytes), CDX2 (a marker expressed in posterior endoderm and gut cells), and SOX2 (a marker expressed in antrum) (supplemental online Fig. 5b). None of these markers were detected in cells from either the PP or PDEC stages, supporting our interpretation that the CFTR/\(\mathrm{SOX9}^+\)/CK19\(^+\) cells are PDECs. In addition, very few insulin-positive cells (pancreatic \(\beta\) cells), glucagon-positive cells (\(\alpha\) cells), or amylase-positive cells (exocrine cells) were detected in HESCs-derived PDEC populations (supplemental online Fig. 5c), suggesting that the vast majority of cells differentiate toward PDECs, using our current strategy.

iPSCs have been derived from patients with CF, most of whom carry the common delF508 mutation [7–10]. We focused instead on patients with CF who were diagnosed specifically with pancreatic disease, and obtained fibroblasts from a patient diagnosed with severe pancreatic insufficiency (Weill Cornell cystic fibrosis patient [WCCF]), who is a compound heterozygote: one allele has a deletion of an adenine residue at nucleotide 444 in exon 4, which results in a frameshift, and a second allele carries a guanine-to-adenine substitution at nucleotide 1812-1 in intron 11, which results in an mRNA splicing defect in the CFTR gene [1812-1G-A]. In addition, fibroblasts were collected from an age-matched control patient (WCC). To induce reprogramming, the fibroblasts were transduced with a lentivirus encoding the transcription factors OCT4, SOX2, KLF4, and cMYC [20]. After 4 weeks, colonies were picked based on morphological resemblance to hESC colonies, and were expanded. After three passages, the iPSC clones were examined by immunocytochemistry using antibodies recognizing pluripotency markers, including NANOG, SOX2, SSEA4, TRA-1-60, TRA-1-81, and the substrate of alkaline phosphatase (Fig. 3A). iPSC lines were evaluated by qRT-PCR experiments to further confirm the expression of endogenous pluripotency markers, including OCT3/4, NANOG, SOX2, GDF3, and REX1 (supplemental online Fig. 6a). The mutant alleles of CF-iPSCs were validated using genomic DNA sequencing (Fig. 3B); qRT-PCR analyses confirmed the silencing of viral transgenes in iPSCs (supplemental online Fig. 6bB) [21]. Finally, all iPSCs were documented to maintain a normal karyotype (supplemental online Fig. 6c).

The key characteristic of iPSCs is pluripotency, which is the ability to differentiate into cells representing each of the three germ layers. We used both in vitro differentiation and in vivo differentiation to test pluripotency. According to in vitro differentiation, the iPSCs were directed successfully to differentiate into TJU1\(^+\) neurons (ectoderm), SOX17\(^+\)/FOXA2\(^+\) DE, and troponin T\(^+\) cardiomyocytes (mesoderm) (supplemental online Fig. 6d). Regarding in vivo differentiation, iPSCs were transplanted subcutaneously into nude mice. Outgrowths formed as soon as 1 month after injection, and typically after 6–8 weeks. The grafts were removed and examined by hematoxylin and eosin staining. Cells or tissues of three germ layers were readily detected, including glandular structures (endoderm), muscle and cartilage (mesoderm), and nerve fibers and pigmented epithelium (ectoderm) (supplemental online Fig. 6e).

The directed differentiation protocol established using hESCs (Fig. 1A) was next applied to control iPSCs (WCC-iPSCs) and CF-iPSCs (WCCF-iPSCs). Both iPSC lines were successfully differentiated into SOX17\(^+\)/FOXA2\(^+\) DE (supplemental online Fig. 3d). After
another 10 days of combined treatment with chemicals and growth factors, more than 80% of the cells expressed the PP marker PDX1+ (Fig. 3C). Two weeks later, more than 80% of WCC-iPSC- and WCCF-iPSC-derived populations stained positively using CK19 and SOX9 antibodies (Fig. 3D, 3E). In addition, the iPSC-derived cells showed increased expression of other PDEC markers, including CFTR, CK19, CAII, and BEST1, shown by qRT-PCR (Fig. 3F). Both immunocytochemistry and qRT-PCR data suggested that WCC-iPSCs and WCCF-iPSCs show comparable capacities to differentiate into PDECs.

The iPSC-derived PDECs were infected with a lentivirus containing the CFTR promoter directing expression of the puromycin-resistance gene to enrich the CFTR+ cells. A CFTR antibody was used to detect expression in the enriched iPSC-derived PDECs (Fig. 4A). In the PDECs derived from control WCC-iPSCs (WCC-PDECs), CFTR protein was detected on cell membranes. However, no CFTR protein was detected in PDECs derived from WCCF-iPSCs (WCCF-PDEC) (Fig. 4A). Western blot experiments further confirmed the expression of mature CFTR band C in PDECs derived from control iPSCs but not WCCF-iPSCs (Fig. 4B).

Under physiologic conditions, CFTR functions as a transmembrane anion channel and transports Cl⁻ and other anions across PDEC membranes [22]. As a result of CFTR mutations, water does not diffuse out of PDECs into the mucus layer, leading to pathologic viscous epithelial secretions. The resultant exocrine fluid thickens in the proximal pancreatic ducts, leading to their obstruction [23]. We used an iodine efflux assay to indirectly measure the Cl⁻ channel activity as a functional readout for CFTR activity. The platform uses a combined Cl⁻/pH fluorescence sensor protein (ClopHensor), in which the cyan-to-red ratio is differentially sensitive to intracellular Cl⁻ ([Cl⁻]i) or intracellular I⁻ ([I⁻]i), to monitor Cl⁻ channel activity [19]. The lentivirus vector used to select for puromycin-resistant PDECs also contains a CMV promoter that drives expression of the ClopHensor protein.

To validate the ClopHensor vector, enriched ClopHensor-expressing WCC-PDECs were cultured in 138 mM I⁻ buffer for 20 minutes to increase [I⁻]. As expected, the cyan-to-red ratio of cells loaded with I⁻ buffer decreased compared with cells cultured in I⁻-free control conditions (Fig. 5A, 5B). Cells were then moved to culture in I⁻-free buffer to monitor I⁻ efflux. A time-course experiment showed that the cyan-to-red ratio increased

Figure 5. PDECs derived from induced pluripotent stem cells that were derived from patients with cystic fibrosis and diagnosed with severe pancreatic insufficiency show impaired Cl⁻ channel activity. Representative single-cell images of WCC-PDECs in the absence (A) or in the presence (C) of CFTRi in the I⁻ efflux assay. Scale bars = 0.8 μm. Quantification of cyan-to-red ratio of WCC-PDECs (B) in the absence or (D) in the presence of CFTRi in the I⁻ efflux assay. Blue dots represent the cyan-to-red ratio of the cells loaded in control buffer after 20 minutes. Brown dots represent the cyan-to-red ratio of the cells loaded in 138 mM I⁻ buffer after 20 minutes. Green dots represent the cyan-to-red ratio of the cells loaded in control buffer after an additional 20 minutes. Orange dots represent the cyan-to-red ratio of the cells loaded first in 138 mM I⁻ buffer for 20 minutes and I⁻-free buffer for an additional 20 minutes. Representative figures (E) and quantification (F) of cyan-to-red ratio of WCCF-PDECs. The data represent 30 cells from 3 independent experiments. Data are presented as mean ± SD; p values were calculated by unpaired two-tailed Student t test. *, p < .05; **, p < .01. Abbreviations: CFTRi, cystic fibrosis transmembrane conductance regulator inhibitor 172; n.s., no significant difference; PDEC, pancreatic ductal epithelial cell; WCC, Weill Cornell control patient; WCCF, Weill Cornell cystic fibrosis patient.
when cells were moved to $I^-_\text{free}$ buffer and became stable after 20 minutes (supplemental online Fig. 7a). Thus, an endpoint assay at 20 minutes after $I^-_\text{free}$ culture was used in all future experiments (supplemental online Fig. 7b). Together, the data suggest that the $I^-_\text{efflux}$ assay faithfully reflected the CFTR $C_l^-$ channel activity, cells were treated with CFTR inhibitor 172 during the 20-minute $I^-_\text{efflux}$ assay. The cyan-to-red ratio of cells treated with this CFTR inhibitor failed to return to the control level (Fig. 5C, 5D). Together, assay. The cyan-to-red ratio of cells treated with this CFTR inhibitor failed to return to the control level (Fig. 5C, 5D). Together, the data suggest that the $I^-_\text{efflux}$ assay faithfully reflects the CFTR $C_l^-$ channel activity, and that WCC-PDECs have functional CFTR $C_l^-$ channels. In contrast to WCC-PDECs, the cyan-to-red ratio in WCCF-PDECs did not return to baseline, suggesting that WCCF-PDECs do not have functional CFTR protein (Fig. 5E, 5F).

**DISCUSSION**

We report an efficient strategy to differentiate human iPSCs into PDECs expressing functional or mutant CFTR. In addition, we established a platform to purify the derived PDECs. After puromycin-based purification, more than 98% of hESC-derived cells expressed functional CFTR protein. Since most hESC/iPSC-derived cells are embryonic or fetal-like, the PDECs we have studied are likely also phenotypically closer to embryonic or fetal, rather than adult, PDECs. However, many studies have successfully used hESC/iPSC-derived cells to model human diseases, including Parkinson’s disease [24, 25], Alzheimer’s disease [26], and diabetes [27]. Moreover, most pancreatic insufficiency cases are diagnosed at birth [28]. Thus, the embryonic or fetal-like phenotype of hESC/iPSC-derived PDECs should not obviate their application to model the pancreatic defect of patients with CF.

Deriving mature functional cells is a common challenge in the hESC/iPSC differentiation field. Here, we identified BMP4 and VPA to significantly increase the differentiation efficiency toward the PDEC lineage. In addition, the PDECs derived after BMP4 and VPA treatment expressed functional CFTR channel (Fig. 4); in contrast, the PDECs derived from spontaneous differentiation were far fewer and had relatively lower CFTR protein expression levels (supplemental online Fig. 8). Because the PDECs derived from directed differentiation had a functional CFTR channel (supplemental online Fig. 2), it appears that BMP4 and VPA treatment not only increases the differentiation efficiency but also promotes PDEC maturation.

Recently, significant progress has been made in using hESC-derived epithelial cells to model cystic fibrosis. Several groups have successfully generated lung epithelial cells and cholangiocytes from CF-iPSCs, which showed decreased expression of CFTR protein, and impaired $C_l^-$ channel activity [5–11]. The pancreas is among the first and most seriously affected organs impacted in patients with CF. Here, we established an efficient strategy to derive functional PDECs, which will facilitate study of the multiorgan defects for certain mutations.

**CONCLUSION**

In this study, we established a platform to monitor the CFTR activity of derived human PDECs at the single-cell level. The PDECs derived from CF-iPSCs showed decreased CFTR protein expression levels and impaired $C_l^-$ channel activity, which recapitulates the functional defects of PDECs in patients with CF. This platform can be easily adapted in a high-throughput manner to evaluate drug candidates for their ability to correct functional defects of CF-PDECs. In the future, it will be feasible to develop a battery of iPSC lines carrying a spectrum of CF mutations and use the established platform to identify drug candidates that correct functional defects of PDECs carrying specific mutations.

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**AUTHOR CONTRIBUTIONS**

S.S.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript. T.Z., C.L.R., S.-Y.T., M.C., S.A., X.L., and J.H.: collection and/or assembly of data; T.E.: conception and design, manuscript writing, final approval of manuscript; S.C.: conception and design, financial support, data analysis and interpretation, manuscript writing, final approval of manuscript.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

The authors indicated no potential conflicts of interest.

**REFERENCES**

1 Rogers CS, Stoltz DA, Meyerholz DK et al. Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. Science 2008; 321:1837–1841.

2 Sun X, Sui H, Fisher JT et al. Disease phenotype of a ferret CFTR-knockout model of cystic fibrosis. J Clin Invest 2010;120: 3149–3160.

3 Wilke M, Buijs-Offeman RM, Aarbiou J et al. Mouse models of cystic fibrosis: Phenotypic analysis and research applications. J Cyst Fibros 2011;10(suppl 2):S152–S172.

4 Inoue H, Nagata N, Kurokawa H et al. iPSC cells: A game changer for future medicine. EMBO J 2014;33:409–417.

5 Mou H, Zhao R, Sherwood R et al. Generation of multipotent lung and airway progenitors from mouse ESCs and patient-specific cystic fibrosis iPSCs. Cell Stem Cell 2012;10: 385–397.

6 Firth AL, Dargitz CT, Qualls SJ et al. Generation of multiciliated cells in functional airway epithelia from human induced pluripotent stem cells. Proc Natl Acad Sci USA 2014;111:E1723–E1730.

7 Wong AP, Bear CE, Chin S et al. Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein. Nat Biotechnol 2012; 30:876–882.

8 Sampaziotis F, Cardoso de Brito M, Madrigal P et al. Cholangiocytes derived from human induced pluripotent stem cells for disease modeling and drug validation. Nat Biotechnol 2015; 33:845–852.
9 Wong AP, Chin S, Xia S et al. Efficient generation of functional CFTR-expressing airway epithelial cells from human pluripotent stem cells. Nat Protoc 2015;10:363–381.
10 Crane AM, Kramer P, Bui JH et al. Targeted correction and restored function of the CFTR gene in cystic fibrosis induced pluripotent stem cells. Stem Cell Rep 2015;4:569–577.
11 Ogawa M, Ogawa S, Bear CE et al. Directed differentiation of cholangiocytes from human pluripotent stem cells. Nat Biotechnol 2015;33:853–861.
12 Borowiak M, Maehr R, Chen S et al. Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. Cell Stem Cell 2009;4:348–358.
13 Chen S, Borowiak M, Fox JL et al. A small molecule that directs differentiation of human ESCs into the pancreatic lineage. Nat Chem Biol 2009;5:265.
14 Haumaitre C, Lenoir O, Scharfmann R. Histone deacetylase inhibitors modify pancreatic cell fate determination and amplify endocrine progenitors. Mol Cell Biol 2008;28:6373–6383.
15 Inada A, Nienaber C, Fonseca S et al. Timing and expression pattern of carbonic anhydrase II in pancreas. Dev Dyn 2006;235:1571–1577.
16 Marsey LL, Winpenny JP. Bestrophin expression and function in the human pancreatic duct cell line, CFPAC-1. J Physiol 2009;587:2211–2224.
17 O’Riordan CR, Lachapelle AL, Marshall J et al. Characterization of the oligosaccharide structures associated with the cystic fibrosis transmembrane conductance regulator. Glycobiology 2000;10:1225–1233.
18 Koh J, Sierra TI, Collins FS. Characterization of the cystic fibrosis transmembrane conductance regulator promoter region. Chromatin context and tissue-specificity. J Biol Chem 1993;268:15912–15921.
19 Arosio D, Ricci F, Marchetti L et al. Simultaneous intracellular chloride and pH measurements using a GFP-based sensor. Nat Methods 2010;7:516–518.
20 Carey BW, Markoulaki S, Hanna J et al. Reprogramming of murine and human somatic cells using a single polycistronic vector [published corrections appear in Proc Natl Acad Sci USA 2009;106:5449; and Proc Natl Acad Sci USA 2009;106:11818]. Proc Natl Acad Sci USA 2009;106:157–162.
21 Sommer CA, Stadtfeld M, Murphy GJ et al. Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. STEM CELLS 2009;27:543–549.
22 Gregory RJ, Cheng SH, Rich DP et al. Expression and characterization of the cystic fibrosis transmembrane conductance regulator. Nature 1990;347:382–386.
23 Nousia-Arvanitakis S. Cystic fibrosis and the pancreas: Recent scientific advances. J Clin Gastroenterol 1999;29:138–142.
24 Nguyen HN, Byers B, Cord B et al. LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. Cell Stem Cell 2011;8:267–280.
25 Cooper O, Seo H, Andrabí S et al. Pharmacological rescue of mitochondrial deficits in iPSC-derived neural cells from patients with familial Parkinson’s disease. Sci Transl Med 2012;4:141ra90.
26 Israel MA, Yuan SH, Bardy C et al. Probing sporadic and familial Alzheimer’s disease using induced pluripotent stem cells. Nature 2012;482:216–220.
27 Hua H, Shang L, Martinez H et al. iPSC-derived β cells model diabetes due to glucokinase deficiency. J Clin Invest 2013;123:3146–3153.
28 Kumar V, Abbas AK, Fausto N et al. (eds). Robbins Basic Pathology. Philadelphia, PA: Saunders/Elsevier, 2007.