Efficient Generation of Small Intestinal Epithelial-like Cells from Human iPSCs for Drug Absorption and Metabolism Studies

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SUMMARY

The small intestine plays an important role in the absorption and metabolism of oral drugs. In the current evaluation system, it is difficult to predict the precise absorption and metabolism of oral drugs. In this study, we generated small intestinal epithelial-like cells from human induced pluripotent stem cells (hiPS-SIECs), which could be applied to drug absorption and metabolism studies. The small intestinal epithelial-like cells were efficiently generated from human induced pluripotent stem cell by treatment with WNT3A, R-spondin 3, Noggin, EGF, IGF-1, SB202190, and dexamethasone. The gene expression levels of small intestinal epithelial cell (SIEC) markers were similar between the hiPS-SIECs and human adult small intestine. Importantly, the gene expression levels of colonic epithelial cell markers in the hiPS-SIECs were much lower than those in human adult colon. The hiPS-SIECs generated by our protocol exerted various SIEC functions. In conclusion, the hiPS-SIECs can be utilized for evaluation of drug absorption and metabolism.

INTRODUCTION

Many drugs are administered orally. Drugs administered in this fashion are absorbed and metabolized in the small intestine. Thus, a model that could evaluate drug absorption and metabolism in the small intestine would be useful for drug discovery. Currently, experimental animals and cancer cell lines are widely used as intestinal models for small intestinal absorption and metabolism studies because primary human small intestinal epithelial cells are difficult to obtain and culture (Di Claudio et al., 2017; Hansen et al., 2000; Shirasaka et al., 2006; Taylor et al., 2010; Theodoropoulos et al., 2003; Yamashita et al., 2002). These existing models, however, do not accurately reflect the human small intestine functions (Gupta et al., 2008; Hartley et al., 2006; Sun et al., 2008). This is because the expression patterns of drug transporters and drug-metabolizing enzymes in the existing models are different from those in the human small intestine (Lu and Li, 2001; Martignoni et al., 2006; Maubon et al., 2007; Nakamura et al., 2002). Therefore, it is difficult to precisely predict the absorption and metabolism of oral drugs by using existing models. An evaluation system whereby a model can evaluate drug absorption and metabolism in the small intestine is thus greatly needed.

Recently, several researchers have reported that intestinal organoids can be differentiated from human pluripotent stem cells in vitro (Forster et al., 2014; Kauffman et al., 2015; Spence et al., 2011; Tamminen et al., 2015). Human intestinal organoids consist of all small intestinal cell types (paneth cells, goblet cells, enterocytes, and enteroendocrine cells). Human intestinal organoids are very attractive cell sources in terms of regenerative medicine. However, it would be difficult to generate a monolayer small intestine model, such as could be used in pharmaceutical research, using these intestinal organoids. On the other hand, several groups have demonstrated that a monolayer small intestine model can be generated from human pluripotent stem cells. Ogaki and co-workers succeeded in generating epithelial-like cells (ELCs) from human pluripotent stem cells in vitro by using (2Z,3′E)-6-bromoindirubin-3′-oxime (BIO) and N-[N-(3,5-difluorophenacyl-L-alanyl)]-(S)-phenylglycine t-buty1 ester (DAPT), but there is room for improvement in terms of the differentiation efficiency (Ogaki et al., 2013, 2015). Kauffman et al. (2013) reported that human induced pluripotent stem cell (iPSC)-derived epithelial-like cells (hiPS-ELCs) form a monolayer showing barrier formation. However, the usefulness of the hiPS-ELCs in pharmaceutical research of oral drugs has not been adequately validated, because the evaluation of small intestinal drug-metabolizing enzymes and drug transporters has not been well characterized.

We previously showed that intestinal epithelial cell differentiation from human iPSCs could be promoted by using WNT3A, epidermal growth factor (EGF), SB431542, and overlaying Matrigel (Negoro et al., 2016; Ozawa...
et al., 2015). Moreover, we succeeded in establishing an intestinal epithelial cell model from human iPSCs that has the potential to be applied in drug absorption and metabolism studies. However, further enhancement of the intestinal epithelial cell differentiation efficiency is required because the percentage of villin 1-positive cells in the hiPS-ELCs was not high enough (approximately 55%). In addition, intestinal epithelial cells are known to have different properties in the small intestine and the colon (Beuling et al., 2012; Walker et al., 2014b, 2014a). For example, it is known that the expression levels of peptide transporter 1 (PEPT1), cytochrome P450 3A4 (CYP3A4), apolipoprotein A4 (APOA4), and apolipoprotein C3 (APOC3) in the small intestine are higher than those in the colon (Berggren et al., 2007; Meier et al., 2007; Walker et al., 2014a). To establish a small intestinal model for oral drug discovery, it is essential to prepare small intestinal epithelial-like cells, not colonic ELCs. Nevertheless, to the best of our knowledge there have been no reports examining whether hiPS-ELCs have the properties of the small intestinal epithelial cells or colonic epithelial cells.

In this study, we developed a highly efficient differentiation protocol of human iPSC-derived small intestinal epithelial-like cells (hiPS-SIECs) by referring to the developmental process of the small intestine and the method of culturing intestinal organoids. In addition, we examined whether human iPSC-derived cells have small intestinal or colonic properties. Finally, we examined the drug absorption and metabolism capacities of hiPS-SIECs.

RESULTS

LY2090314 Treatment Promoted the Intestinal Progenitor Cell Differentiation of Human iPSCs

Activation of the WNT/β-catenin signal is known to be important for the differentiation of cells from definitive endoderm cells to intestinal progenitor cells (Spence et al., 2011). We therefore performed a screen for glycogen synthase kinase 3β (GSK3β) inhibitors, which can activate WNT/β-catenin signaling (Figure 1A). We used BIO and DAPT as controls for intestinal progenitor cell differentiation (Ogaki et al., 2013). As a result of GSK3β inhibitor screening, the expression level of intestinal progenitor cell marker caudal type homeobox 2 (CDX2) was increased in the LY2090314-treated cells (Figure 1B). In addition, the expression levels of the pancreatic progenitor cell marker pancreas and duodenal homeobox 1 (PDX1) and hepatic progenitor marker alpha fetoprotein (AFP) in LY2090314-treated cells were lower than those in definitive endoderm cells (DECs) (Figure 1B). Moreover, the expression levels of CDX2 were increased in a concentration-dependent manner by LY2090314 treatment (Figures S1A and S1B). Consistently, the CDX2 protein expression level was increased by LY2090314 treatment (Figure 1C). To examine the intestinal progenitor cell differentiation efficiency, we examined the percentage of CDX2-positive cells in the human iPSC-derived intestinal progenitor cells by fluorescence-activated cell sorting (FACS) analysis (Figure S1C). The percentage of CDX2-positive cells was approximately 50%. Moreover, immunohistochemical analysis showed that more than 90% of human iPSC-derived intestinal progenitor cells were positive for CDX2 (Figures 1D and S1D). This discrepancy of percentage of CDX2-positive cells might be due to the difference of detection limit between FACS and immunohistochemical analyses. These results suggest that LY2090314 is a GSK3β inhibitor suitable for selective and efficient intestinal progenitor cell differentiation.

Promotion of Intestinal Epithelial Cell Differentiation by WNT3A, R-Spondin 3, Noggin, EGF, IGF-1, SB202190, and DEX Treatments

We next performed a screening experiment to establish an efficient intestinal epithelial cell differentiation protocol, as shown in Figure 2A. The test compounds or cytokines used are known to regulate signals that play important roles in intestinal epithelial cell differentiation, small intestinal stem cell homeostasis, or small intestinal epithelial cell maturation. We used BIO and DAPT as controls for the intestinal epithelial cell differentiation (Ogaki et al., 2013). The gene expression levels of the intestinal epithelial cell markers villin 1 and intestine specific homeobox (ISX) were examined by real-time RT-PCR on day 20. The gene expression levels of villin 1 and ISX were increased in the cells treated with SB202190, dexamethasone (DEX), triiodothyronine (T3), SB431542, EGF, insulin-like growth factor 1 (IGF-1), WNT3A-conditioned medium, and WRN-conditioned medium (Figure 2B). We also found that the combination of WRN (WNT3A, R-spondin 3, Noggin), EGF, IGF-1, SB202190, and DEX resulted in the most efficient intestinal epithelial cell differentiation, judging from the gene expression levels of villin 1 and ISX (Figure 2C). These results suggest that the combination of WRN, EGF, IGF-1, SB202190, and DEX was suitable for efficient intestinal epithelial cell differentiation. In the following experiments, this combination was used.

WNT3A, R-Spondin 3, Noggin, EGF, IGF-1, SB202190, and DEX Treatments Promoted Intestinal Epithelial Cell Differentiation

We optimized the culture period to increase the intestinal epithelial cell differentiation efficiency as shown in Figure 3A. The gene expression levels of villin 1, ISX, and CDX2 in the WRN-, EGF-, IGF-1-, SB202190-, and DEX-treated cells were examined by real-time RT-PCR analysis at day 20, 24, or 28 of differentiation. The gene expression
levels of villin 1, ISX, and CDX2 were the highest at day 28 (Figure 3B). Next, the gene expression levels of the intestinal epithelial cell markers—i.e., villin 1, ISX, CDX2, alanyl aminopeptidase, membrane (ANPEP), and sucrase-isomaltase (SI)—in the hiPS-SIECs (day 28) were compared with those in the human adult small intestine (hereafter small intestine) by real-time RT-PCR analysis. The gene expression levels of villin 1 were similar between the hiPS-SIECs and the small intestine, while the gene expression levels of ISX and ANPEP in the hiPS-SIECs were lower than those
of the small intestine (Figure 3C). The SI expression level of hiPS-SIECs was about 1/62 of that in the small intestine (Figure 3C). We performed immunostaining of E-cadherin and Zonula occludens-1 (ZO-1). Immunohistochemical analysis showed that the hiPS-SIECs were positive for E-cadherin and ZO-1 (Figures S2A and S2B). The percentages of villin 1- and SI-positive cells in the hiPS-SIECs were examined by FACS analysis. The percentage of villin 1- and SI-positive cells was approximately 95% and 88%, respectively (Figure 3D). In addition, two other human iPSC lines, YOW-iPS and HC2-14-iPSCs, were differentiated into intestinal ELCs and then the gene expression levels of intestinal epithelial cell markers (Figure S2C) and villin (Figure S2D). We were able to perform intestinal epithelial cell differentiation at high efficiency in the two other human iPSC lines as well as the human iPSC line, Tic. To further characterize the hiPS-SIECs, we prepared confocal optical cross-sections and performed immunostaining of villin 1. Apical villin 1 localization was confirmed in our hiPS-SIECs (Figure 3E). We also evaluated alkaline phosphatase (ALP) activity of hiPS-SIECs (Figure 3F). The ALP activity was detected in the hiPS-SIECs, whereas it was not confirmed in a negative control, mouse embryonic fibroblast (MEFs) (Figure 3F). In addition, we observed the microvillus of monolayer of the hiPS-SIECs using an electron microscope (Figure 3G). Our hiPS-SIECs exhibited columnar morphology and microvillus structure. We performed immunostaining analysis of chromogranin A (CHGA), lysozyme, and mucin 2 (MUC2) to confirm the existence of enteroendocrine, paneth, and goblet cells, respectively (Figure S2E). We confirmed the existence of other major intestinal epithelial cell types in our model. These results suggest that the intestinal epithelial cell differentiation was promoted by treatment with WRN, EGF, IGF-1, SB202190, and DEX. Although the gene expression levels of PDX1, pluripotent marker SOX2, and lung marker NXX2.1 in hiPS-SIECs were at nearly background levels, the gene expression levels of AFP in the hiPS-SIECs were much higher than those in the small intestine (Figure S2F). Immunohistochemical analysis also showed that the hiPS-SIECs were positive for AFP (Figure S2G). These results suggest that there is still room for improvement in the intestinal differentiation method.
We evaluated whether the hiPS-SIECs have small intestinal or colonic properties. First, the gene expression levels of the small intestinal epithelial cell markers—i.e., apolipoprotein A4 (APOA4), apolipoprotein C2 (APOC2), apolipoprotein C3 (APOC3), fibroblast growth factor 19 (FGF19), and GATA binding protein 4 (GATA4)—were examined by real-time RT-PCR analysis (de Wit et al., 2008; Tsai et al., 2017). The gene expression levels of APOA4, APOC2, APOC3, and FGF19 in the hiPS-SIECs were similar to those in the small intestine (Figure 4A and Table S2). The gene expression levels of the colonic epithelial cell markers—i.e., carbonic anhydrase 1 (CA1), carbonic anhydrase 2 (CA2), solute carrier family 2 member 2 (SLC9A2), solute carrier family 9 member 3 (SLC9A3), and SATB homeobox 2 (SATB2)—were also examined by real-time RT-PCR analysis (de Wit et al., 2008; Tsai et al., 2017). The gene expression levels of CA1, CA2, SLC9A2, SLC9A3, and SATB2 in hiPS-SIECs and the small intestine were lower than those in the colon (Figure 4B and Table S3). In addition, we examined global intestinal gene expression profile by microarray analysis. The intestinal-focused gene expression profile in hiPS-SIECs was similar to that of small intestine rather than that of colon (Figure 4C). These results suggest that the hiPS-SIECs indeed have small intestinal characteristics but not colonic characteristics.
Expression Analysis of Drug-Metabolizing Enzymes and Drug Transporters

Drugs administered orally are known to be metabolized not only in the liver but also in the small intestine. Therefore, we first examined the gene expression levels of the drug-metabolizing enzymes cytochrome P450 family 2 subfamily C member 9 (CYP2C9), cytochrome P450 family 2 subfamily J member 2 (CYP2J2), CYP3A4, UDP glucuronosyltransferase family 1 member A1 (UGT1A1), UDP glucuronosyltransferase family 1 member A3 (UGT1A3), and carboxylesterase 2 (CES2)—by real-time RT-PCR analysis. The gene expression levels of drug-metabolizing enzymes in the hiPS-SIECs were lower than those in the small intestine (Figure 5A and Table S4).

It is known that the small intestine plays an important role in intestinal absorption and excretion of oral drugs, because the drug transporters are highly expressed in the small intestine (Giacomini et al., 2010). In the present study, therefore, we first examined the gene expression levels of the drug transporters expressed on the apical surface of enterocytes, i.e., multidrug resistance protein 1 (MDR1), breast cancer resistance protein (BCRP), peptide transporter 1 (PEPT1), multidrug resistance-associated protein 2 (MRP2), multidrug resistance-associated protein 4 (MRP4), and multidrug resistance-associated protein 6 (MRP6), by real-time RT-PCR analysis. The gene expression levels of PEPT1, MRP2, and MRP6 were similar between the hiPS-SIECs and the small intestine (Figure 5B and Table S5). However, the MDR1 expression levels in the hiPS-SIECs were approximately 66 times lower than those in the small intestine (Figure 5B). Next, the gene expression levels of the drug transporters expressed on the basolateral surface of enterocytes, i.e., multidrug resistance-associated protein 1 (MRP1), multidrug resistance-associated protein 3 (MRP3), multidrug resistance-associated protein 5 (MRP5), organic solute transporter alpha (OSTA), and organic solute transporter beta (OSTB), were examined by real-time RT-PCR analysis. The gene expression levels of OSTA were similar between the hiPS-SIECs and the small intestine (Figure 5C and Table S6). However, the MRP3 and OSTB expression levels in the
hiPS-SIECs were lower than those in the small intestine (Figure 5C).

Because Caco-2 cells, human colorectal adenocarcinoma cells, are often used as a human intestinal epithelial cell model for pharmaceutical research, we compared the gene expression levels of drug-metabolizing enzymes and drug transporters between hiPS-SIECs and Caco-2 cells. The gene expression levels of CYP2J2 and MRP4 in the hiPS-SIECs were similar to those in the Caco-2 cells (Figure S3A; Tables S4 and S5). The gene expression levels of CYP3A4, CES2, BCRP, PEPT1, MRP1, and MRP5 in the hiPS-SIECs were higher than those in the Caco-2 cells (Figure S3A and Tables S4–S6). However, the gene expression levels of CYP2C9, UGT1A1, UGT1A3, MDR1, MRP2, MRP6, MRP3, OSTA, and OSTB in the hiPS-ELCs were lower than those in the Caco-2 cells (Figure S3A and Tables S4–S6). These results might suggest that our hiPS-SIECs resemble to fetal rather than the adult small intestine because the gene expression levels of drug-metabolizing enzymes and several drug transporters such as MDR1 in the hiPS-SIECs were lower than those in the adult small intestine.

### The Drug Absorption and Metabolism Capacities of hiPS-SIECs
Among the various CYPs, CYP3A4 has been shown to be a dominant drug-metabolizing enzyme in the small intestinal epithelial cells (Paine et al., 2006). We evaluated the activity levels of CYP3A4 in hiPS-SIECs. The CYP3A4 activity levels in the hiPS-SIECs were approximately 3-fold higher than those in undifferentiated human iPSCs (Figure S3B). Moreover, CYP3A4 expression in the small intestinal epithelial cells can be induced by various drugs (Glaeser et al., 2005; Kolars et al., 1992; Theodoropoulos et al., 2003). However, the use of experimental animal models and cancer cell lines cannot provide an accurate evaluation of the CYP3A4 induction in human small intestinal epithelial cells (Hewitt et al., 2007). In the present study, therefore, to examine the CYP3A4 induction potency of the hiPS-SIECs, these cells were treated with either of two CYP3A4 inducers, 1α,25-dihydroxyvitamin D3 (V3D) and rifampicin (RIF), then the gene expression levels of CYP3A4 were examined by real-time RT-PCR analysis. We used iPSCs as negative controls for a CYP3A4 induction

### Figure 5. Expression Analysis of Drug-Metabolizing Enzymes and Drug Transporters

(A) The gene expression levels of drug-metabolizing enzymes in the human iPSC (Tic)-derived small intestinal epithelial-like cells (hiPS-SIECs), human adult small intestine (Small Intestine), and human adult colon (Colon) were measured by real-time RT-PCR analysis.

(B and C) The gene expression levels of apical transporters (B) and basolateral transporters (C) in the hiPS-SIECs, small intestine, and colon were examined by real-time RT-PCR analysis. On the y-axis, the gene expression levels in the small intestine were taken as 1.0. All data are presented as means ± SEM of three independent experiments. Statistical significance was evaluated by one-way ANOVA followed by Tukey’s post hoc test (p < 0.05). Groups that do not share the same letter are significantly different from each other. See also Figure S3.
test (Figure S3C). The results showed that the CYP3A4 expression levels were increased by RIF or VD3 treatment in the hiPS-SIECs (Figure 6A). It is also known that CES2 is highly expressed in the small intestine (Imai et al., 2006). CES2 contributes to the pharmacokinetics of oral drugs having an ester structure (Imai et al., 2006; Wang et al., 2011). However, the expression levels of CES2 in Caco-2 cells are much lower than those in the human adult small intestine (Imai et al., 2005). Therefore, we also evaluated the activity levels of CES2 in hiPS-SIECs. We confirmed that the CES2 activity levels in the hiPS-SIECs were higher than those in Caco-2 cells (Figure S3D), and the CES2 activity in the hiPS-SIECs was significantly inhibited by loperamide (a CES2 inhibitor) (Figure 6B). These results suggest that hiPS-SIECs could potentially be used to evaluate the CYP3A4 induction test and CES2 activity.

To examine whether the hiPS-SIECs could be applicable to drug permeability studies, we evaluated barrier function in the hiPS-SIECs monolayer by transepithelial electrical resistance (TEER) measurements, Lucifer yellow (LY) and fluorescein isothiocyanate-dextran, average molecular weight 3,000–5,000 (FD4) permeability tests. We used iPSCs and Caco-2 cells as negative controls and positive controls, respectively, for TEER, LY, and FD4 permeability tests (Figures S4A–S4C). The TEER value in the hiPS-SIECs monolayer was approximately 656 $\mu$V cm$^{-2}$ (Figure 6C), indicating that a tight junction was formed. The TEER value in the Caco-2 cells monolayer was approximately 954 $\mu$V cm$^{-2}$ (Figure S4A). It is known that the TEER value in the
Caco-2 cells monolayer is higher than those in the human small intestine (Ayejunie et al., 2014). Thus, the hiPSS-LSCs monolayer would be more suitable for evaluating the absorption of hydrophilic drugs than the Caco-2 cells monolayer. The TEER value was significantly decreased by treatment with capric acid (C10, an absorption-enhancing agent) (Figure 6C). The LY and FD4 apparent membrane permeability coefficient (Papp) values were increased by treatment with C10 (Figures 6D and S4D–S4F). These results suggest that the hiPSS-SIECs could be applicable to drug permeability studies.

MDR1 is known to play an important role in the intestinal absorption and excretion of various drugs, such as digoxin and verapamil. We performed an apical-to-basolateral MDR1 transport assay in the hiPSS-SIECs monolayer by using rhodamine123 (an MDR1 substrate). We used iPSCs and Caco-2 cells as negative controls and positive controls, respectively, for MDR1 transport assay (Figure S4G). The Papp value of rhodamine123 in the hiPSS-SIECs was significantly increased by cyclosporine A (an MDR1 inhibitor) (Figures 6E and S4H). PEPT1 mediates the absorption of various drugs with a peptide-like structure, such as cephalaxin and valaciclovir. We performed a peptide-uptake analysis of a fluorescently labeled tripeptide (D-Ala-Leu-Lys-AMCA, a PEPT1 substrate). At first we prepared confocal optical cross-sections and performed immunostaining of PEPT1. Apical PEPT1 localization was confirmed in our hiPSS-SIECs (Figure S4I). We confirmed that E-cadherin-positive hiPSS-SIECs could take up D-Ala-Leu-Lys-AMCA (Figure 6F). Moreover, this uptake was inhibited by captopril (a PEPT1 inhibitor). Similarly, the Papp value of glycylsarcosine (a PEPT1 substrate) in the hiPSS-SIECs was significantly decreased by captopril (Figure S4I). These results suggest that hiPSS-SIECs have barrier ability and could be applied to the development of an absorbance-accelerating agent. In addition, intestinal absorption and excretion through transporters including MDR1 and PEPT1 may be accurately predicted by using hiPSS-SIECs.

DISCUSSION

The aim of this study was to develop a method for generating small intestinal, but not colonic, ELCs from human iPSCs for the evaluation of drug absorption, metabolism, and excretion. To this end, we performed screening experiments using various compounds or cytokines under the two-dimensional culture condition. We also examined the drug absorption and metabolism capacities of hiPSS-SIECs.

LY2090314 was a suitable GSK3β inhibitor for intestinal progenitor cell differentiation (Figure 1). It is known that a high concentration of WNT3A/FGF4 or BIO/DAPT can promote intestinal progenitor cell differentiation (Ogaki et al., 2013; Spence et al., 2011). We found that LY2090314 treatment could efficiently promote intestinal progenitor cell differentiation as well as BIO/DAPT. Compared with the protocol that uses a high concentration of WNT3A/FGF4, our protocol decreased the cost of intestinal progenitor cell differentiation. It is known that BIO exhibits various off-target effects, such as the inhibition of JAK/STAT signals (Liu et al., 2011). Because JAK/STAT signals are necessary for intestinal stem cell proliferation (Lin et al., 2010), LY2090314 might be a more suitable compound for the promotion of intestinal progenitor cell differentiation as compared with BIO.

We demonstrated that the combination of WRN, EGF, IGF-1, SB202190, and DEX could efficiently promote intestinal epithelial cell differentiation (Figures 2 and 3). Because it is known that WNT3A, R-spondin 3, Noggin, EGF, and SB202190 are necessary for intestinal organoid maintenance (Sato et al., 2011), intestinal stem cells might have appeared among our hiPSS-SIECs. It has been reported that IGF-1 promotes the proliferation of small intestinal epithelial cells, while DEX promotes maturation in neonatal rat small intestinal epithelial cells (Booth et al., 1995; Leeper et al., 1998; McDonald and Henning, 1992). Thus, IGF-1 and DEX treatments would also have promoted intestinal epithelial cell differentiation. In the future it will be necessary to clarify the mechanism of our intestinal epithelial cell differentiation method using WRN, EGF, IGF-1, SB202190, and DEX.

In this study, we succeeded in generating small intestinal, but not colonic, ELCs from human iPSCs (Figure 4). Recently, Munera et al. (2017) reported that the colonic organoid differentiation from human pluripotent stem cells requires activation of transient bone morphogenetic protein (BMP) signals. Thus it might be important to suppress the BMP signal to perform efficient small intestinal differentiation. Because we used Noggin, which can suppress the BMP signal during the small intestinal epithelial cell differentiation, the small intestinal epithelial cell differentiation would have been successfully performed.

The gene expression levels of most drug transporters were similar between the hiPSS-SIECs and the adult small intestine (Figures 5 and 6). However, the gene expression levels of CYP3A4 and MDR1 in hiPSS-SIECs were still lower than those in the small intestine. These phenomena were also observed in other studies (Iwao et al., 2015; Ogaki et al., 2015; Ozawa et al., 2015). Therefore, further small intestinal epithelial cell maturation studies are needed. In our previous report, hepatic maturation of the human iPSC-derived hepatocyte-like cells was enhanced by gene transfer technology (Nakamori et al., 2016; Takayama et al., 2012b, 2012a). This technology might also be useful for improvement of the small intestinal epithelial cell maturation.
Recently, Vernetti et al. (2017) showed the possibility of evaluating systemic pharmacokinetics by using the Organs-on-a-Chip system, which consists of various types of cells in the body including intestinal organs. Organs-on-a-Chip would be an attractive model for systemic pharmacokinetic study. Although it is difficult to obtain various types of cells from the same donor, human iPSC-derived cells would be able to provide various types of cells from the single donor. It is known that there are large inter-individual differences in drug-metabolizing capacity due to the variety of genetic background (Ingelman-Sundberg, 2001; Zhou, 2009). Thus, human iPSC-derived cells, but not biopsy-derived organoids, might be the most appropriate model for the Organs-on-a-Chip system.

In this study, we have succeeded in generating small intestinal epithelial cells from human iPSCs for use in drug absorption and metabolism tests. We believe that our hiPSC-SIECs model would greatly accelerate the safe and efficient discovery and development of novel drugs.

EXPERIMENTAL PROCEDURES

Human iPSC Culture

Three human iPSC lines, Tic (provided by Dr. A. Umezawa, National Center for Child Health and Development), YOW-iPSCs, and HC2-14-iPSCs (Takayama et al., 2014) were maintained on a feeder layer of mitomycin C-treated MEFs (Merck Millipore) with ReproStem medium (ReproCELL) supplemented with 10 ng/mL FGF2 (Katayama Chemical Industries).

In Vitro Differentiation

Before the initiation of small intestinal epithelial cell differentiation, human iPSCs were dissociated into clumps by using dispase (Roche) and plated onto growth factor reduced BD Matrigel Basement Membrane Matrix (BD Biosciences). These cells were cultured in the MEF-conditioned medium for 2–3 days. The differentiation protocol for the induction of DEC was described previously (Takayama et al., 2014). In brief, for the definitive endoderm cell differentiation, human iPSCs were cultured for 4 days in WNT3A-expressing cell (ATCC; CRL2647)-conditioned RPMI1640 medium (Sigma-Aldrich) containing 100 ng/mL activin A (R&D Systems), 1× GlutaMAX (Thermo Fisher Scientific), 0.2% fetal bovine serum (FBS), and 1× B27 Supplement Minus Vitamin A (Thermo Fisher Scientific). For the induction of intestinal progenitor cells, the DEC were cultured for 4 days in an intestinal differentiation medium, DMEM-High Glucose medium (FUJIFILM Wako) containing 10% knockout serum replacement (Thermo Fisher Scientific), 1% non-essential amino acid solution (NEAA; Thermo Fisher Scientific), penicillin/streptomycin (P/S; Nacalai Tesque) and 1× GlutaMAX supplemented with 20 nM 019.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and six tables and can be found with this article online at https://doi.org/10.1016/j.stemcr.2018.10.019.

AUTHOR CONTRIBUTIONS

K.T. and H.M. conceived and supervised the study; R.N. designed the experiments; R.N., K.T., K.K., K. Harada, F.S., K. Hirata, and H.M. analyzed the data; R.N., K.T., and H.M. wrote the manuscript.

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