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ヒトiPS細胞由来腸管上皮細胞の特性と病態モデルとしての有用性

Characteristics of small intestinal epithelial cells derived from human induced pluripotent stem cells and usefulness as an in vitro model for gastrointestinal disease.

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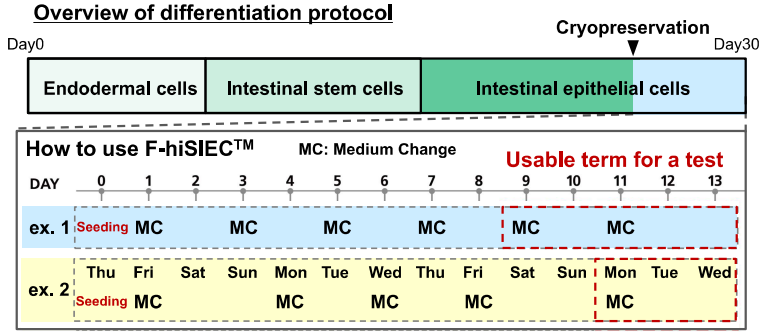
Abstract

【背景】小腸は、食品や医薬品の吸収・代謝を担う消化器官としての役割に加え、クローン病など炎症性腸疾患の好発臓器としても知られている。現在、代替モデルとしてCaco-2細胞や実験動物が用いられているが、各種代謝酵素/トランスポーターの発現量の違いや種差により、ヒト生体小腸との外挿性が低いことが問題となっている。そこで我々はヒトiPS細胞由来腸管上皮細胞（F-hiSIEC™）を開発し、この細胞を用いてヒト生体に近い性質を有するin vitro細胞アッセイモデルの構築を試みた。
【方法】既報（Kabeya, et al. Drug Metab. Pharmacokinet. 2020）を基に、ヒトiPS細胞から腸管上皮細胞への分化誘導方法を確立した。開発したF-hiSIEC™について、種々の特性を評価した。
【結果】F-hiSIEC™は、トランスポーター及び代謝酵素のmRNA発現がヒト小腸とほぼ同等であり、主要なトランスポーターや代謝酵素について安定した活性を示した。また、本細胞は、腸管上皮に存在することが知られている杯細胞・内分泌細胞・M細胞等などを含んでいた。そこで、F-hiSIEC™に存在するM細胞の機能を評価したところ、管腔側から血管側へ粒子の移行が観察され、M細胞が機能していることが示された。次に本細胞を用いて炎症性腸疾患モデルの検討を行った。血管側に炎症性サイトカインを添加し培養すると、経上皮電気抵抗値とMUC2の発現量が低下し、各種炎症性サイトカインの発現量が増加した。また、炎症を抑制することが知られている腸内細菌代謝物を管腔側に併用したところ、上記の炎症反応が抑制された。
【結論】本細胞はヒト小腸に近い性質を有し、M細胞を介したナノ粒子、マイクロプラスチックの吸収やIBD、リーキーガットのヒト小腸in vitroモデルとして、有用なツールとなることが期待される。

The small intestine is known not only for its role as a digestive organ, but also as a favorite organ for inflammatory bowel diseases (IBD). Caco-2 cells and animals are used as alternative models, but the problem is poor extrapolation to the human biological small intestine due to different gene expression levels and species differences. Therefore, we developed human iPS cell-derived intestinal epithelial cells (F-hiSIEC™) and we attempted to construct an in vitro cell assay model with properties similar to those of human organisms.
F-hiSIEC™ shows the physiological characteristics similar to those of the human small intestine. Interestingly, F-hiSIEC™ contains goblet cells, endocrine cells, M cells, etc., which are known to exist in the intestinal epithelium. Therefore, we evaluated the function of M cells present in F-hiSIEC™. As a result, particle migration from the luminal side to the vascular side was observed. Next, we investigated the development of an IBD model using F-hiSIEC™. When inflammatory cytokines were added to the model, TEER and the MUC2 expression was decreased, and the expression of various inflammatory cytokines was increased. When intestinal bacterial metabolites were added to the model in combination with the cytokine stimulation, the inflammatory responses described above were suppressed.
In conclusion, these cells have properties similar to the human small intestine, and they are expected to be a useful tool for M cell-mediated absorption of nanoparticles and microplastics, and as an in vitro model of IBD and leaky gut in the human small intestine.

Method

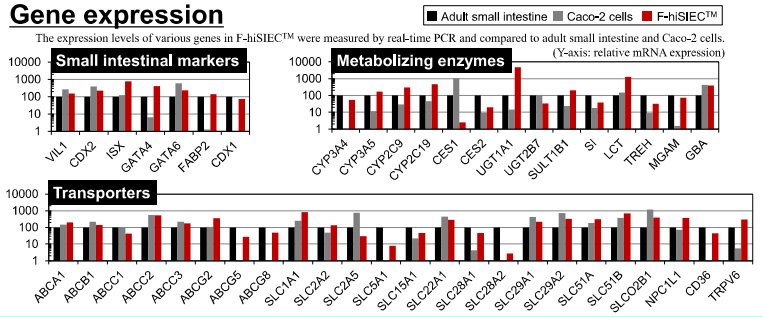
Overview of differentiation protocol



F-hiSIEC™ differentiate into mature small intestinal cells by culturing for 9-11 days after cell seeding.

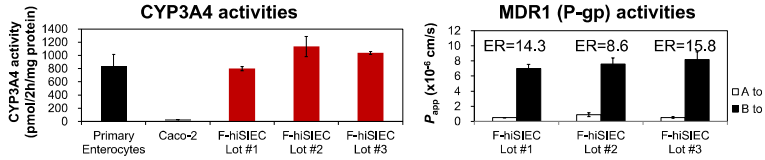
Characteristics of F-hiSIECTM

Gene expression



F-hiSIEC™ showed a gene expression pattern similar to that of human small intestine.

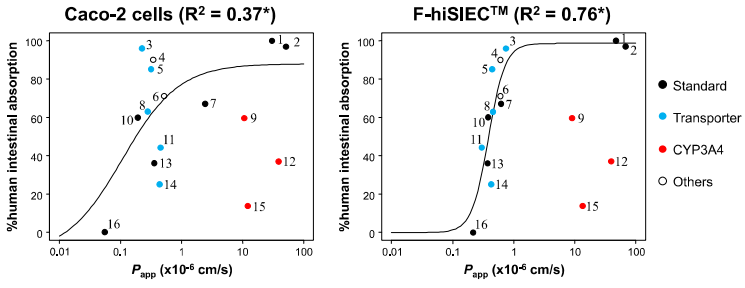
Activities of metabolizing enzyme and transporter



Each cells were incubated with 5 μM midazolam for 2h at 37 °C. CYP3A4 in F-hiSIEC™ was measured with F-hiSIEC™ Assay Medium.
Human Enterocytes (purchased from In Vitro ADMET Laboratories, Inc.) were used as primary small intestinal cells.
10 μM digoxin was used as a substrate . Additionally, HBSS containing 10 mM HEPES (pH 7.4) was used as a transport buffer. After the preincubation, the transport buffer containing the substrate was added to the apical or basal chambers, and the cells were incubated at 37°C for 60 min.

F-hiSIEC™ showed CYP3A4 activity comparable to that of human primary enterocytes. CYP3A4 activities and P-gp activities of F-hiSIEC™ showed good reproducibility.

Drug permeability assay

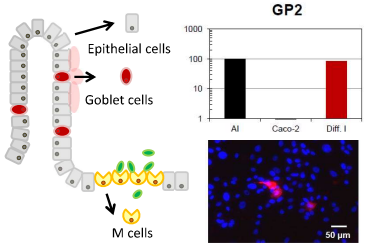


1. Antipyrine , 2. Caffeine, 3. Cephalexin, 4. Acebutolol, 5. Ribavirin, 6. Metformin, 7. Hydrochlorothiazide, 8. Indinavir, 9. Verapamil, 10. Enalapril, 11. Erythromycin, 12. Midazolam, 13. Sulpiride, 14. Lisinopril, 15. Tacrolimus, 16. Lucifer yellow.
*R^2 scores were determined exclude CYP3A4 substrates (9, 12 and 15).

There was a correlation between P_{app} and human intestinal absorption.

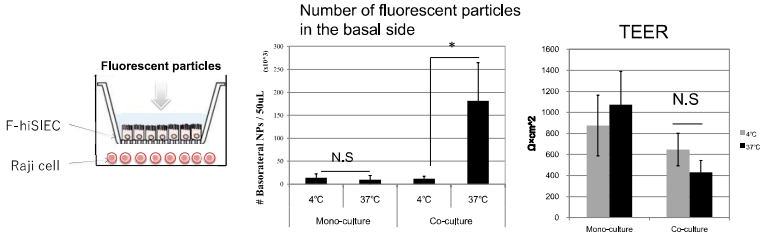
Micro particle absorption via M cells

M cell of F-hiSIEC



- **Microfold cells (or M cells) located in the gut-associated lymphoid tissue (GALT) of the Peyer's patches in the small intestine.**
  - M cells have the unique ability to take up antigens from the lumen of the small intestine via endocytosis, phagocytosis, or transcytosis.
  - M cells are expected to be a target for drug delivery systems of nanoparticulated drugs.
- **F-hiSIEC contained M cells, but it was unclear whether they were functional.**

M cells absorption assay



N=4 *$p \leqq 0.05$

Five days after cell seeding, the cells were cultured on cell culture inserts, and Raji cells (lymphoblast-like cells) were co-cultured on the basal side of the cell culture inserts. On day 10, fluorescent particles (0.2μm) were added to the apical side and incubated for 2h. The number of fluorescent particles passed through to the basal side was counted by flow cytometry.

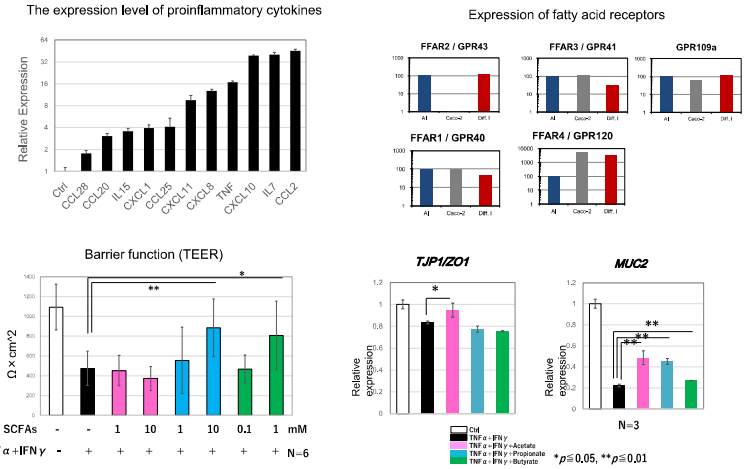
Co-culture of F-hiSIEC with Raji cells resulted in the absorption of fluorescent particles (0.2μm). The transport of the fluorescent particles was temperature-dependent, suggesting that it was transcytosis of M cells. In addition, there was no change in the barrier function by Raji cell co-culture.

F-hiSIEC™ can be used as the evaluation model for drug absorption through M cells.

Inflammatory Bowel Disease model

- **Inflammatory bowel disease is the collective term for Crohn's disease and ulcerative colitis, and the number of patients is increasing yearly. IBD is a multifactorial disease associated with genetic and environmental factors.**
- **We attempted to construct an inflammatory bowel disease model using these cells.**

Influence of Inflammatory Cytokines and SCFAs on IBD model



On day 8 of cell seeding, inflammatory cytokines (100 ng/mL TNFα, 50 ng/mL IFNγ) were added to the basal side of the cell culture insert and short-chain fatty acids to the Apical side. Expressions of cytokines ,TEER and genes expression were evaluated 24 hours after addition.

The decrease of barrier function and expression of the TJP1/ZO-1 and MUC2 genes with TNFα and IFNγ treatment were partially recovered by combination treatment of SCFAs.

The F-hiSIEC-based evaluation system allowed us to reproduce the effects of inflammation and SCFAs on barrier function as in the human small intestine.

Conclusion : F-hiSIEC™ has properties similar to the human small intestine, and they are expected to be a useful tool for M cell-mediated absorption of nanoparticles and microplastics, and as an in vitro model of IBD and leaky gut in the human small intestine.