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ヒトiPS細胞由来腸管上皮細胞の特性と消化管毒性評価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 toxicity evaluation

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ぜひ毒性研究に関わる皆様の
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Abstract

【背景】医薬品・機能性食品の多くは、経口摂取により小腸から吸収される。そのため、小腸における化合物の吸収・代謝を予測することは、毒性を予測する上で重要である。また、小腸は恒常的に増殖と分化を繰り返しているため、様々な化合物の影響を受けやすく、毒性の標的臓器となることが知られている。そこで、我々はヒトiPS細胞から腸管上皮細胞を分化誘導し、既存の小腸代替モデル（Caco-2細胞・実験動物等）よりもヒト生体に近い性質を示す*in vitro*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。開発した細胞製品F-hiSIEC™について、種々の特性を評価した。

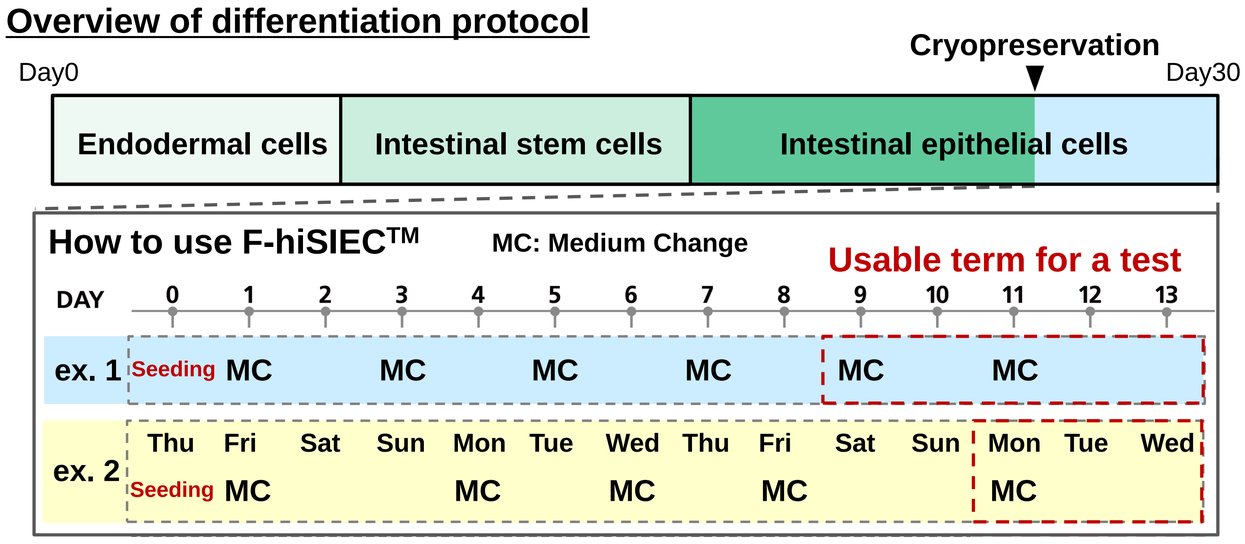
【結果】本細胞は、トランスポーター及び代謝酵素のmRNA発現がヒト小腸とほぼ同等であり、主要なトランスポーターや代謝酵素について安定した活性を示した。また、本細胞を用いた薬物透過試験により、腸管吸収性をCaco-2細胞よりも高い精度で予測できることが示された。次に消化管毒性を評価する目的で、ヒトで消化管障害を誘発するとされる抗がん剤（5-FU）とそのプロドラッグ（5'-DFUR、カペシタビン）を本細胞に作用させたところ、バリア機能やEdU陽性細胞数等を指標に、消化管毒性の程度を評価することができた。また、本細胞は成熟過程で杯細胞・内分泌細胞・M細胞等の腸管上皮に存在する6種の細胞へと分化することがわかっている。そこで、腸管上皮の分化過程に異常をきたすとされるγセクレターゼ阻害剤を作用させると、杯細胞マーカーの発現上昇が見られ、腸管上皮の異常分化を評価できることが示された。

【結論】本細胞はヒト小腸に近い性質を有し、医薬品・有害物質の動態や消化管毒性を評価するヒト小腸*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。

F-hiSIEC™ is a human iPS cell-derived small intestinal epithelial-like cell which shows the physiological characteristics similar to those of the *in vivo* human small intestine such as CYP3A4 activity and expression of transporter genes. To extend the applicability of F-hiSIEC™ to gastrointestinal (GI) toxicity assessment, several types of reference drugs were examined in this study. The results of the present study show an *in vitro* system using F-hiSIEC™ can be a useful model for evaluating GI toxic agents and effects of drug candidates on differentiation of intestinal epithelium.

Method

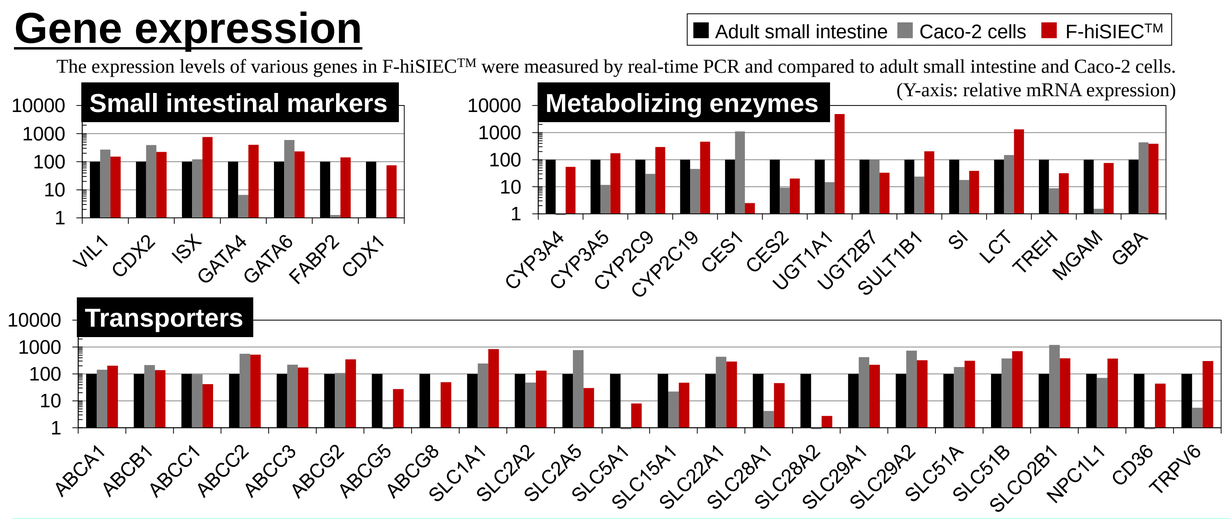
Overview of differentiation protocol



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

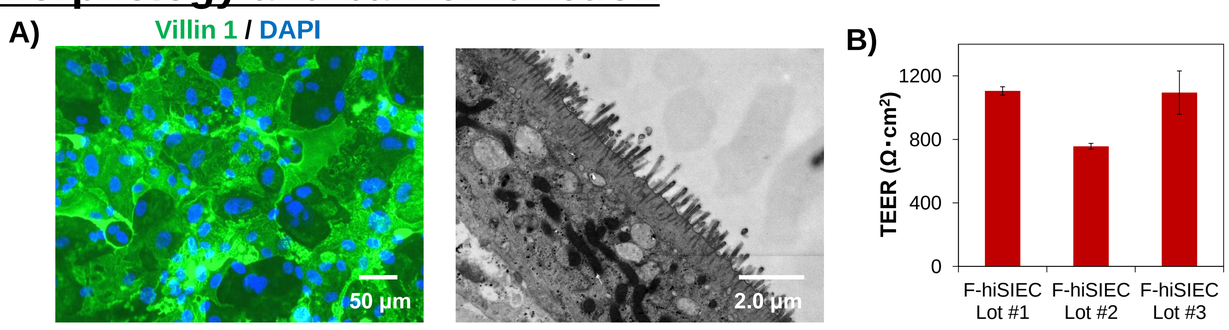
Characteristics of iPSC-derived small intestinal epithelial-like cells

Gene expression



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

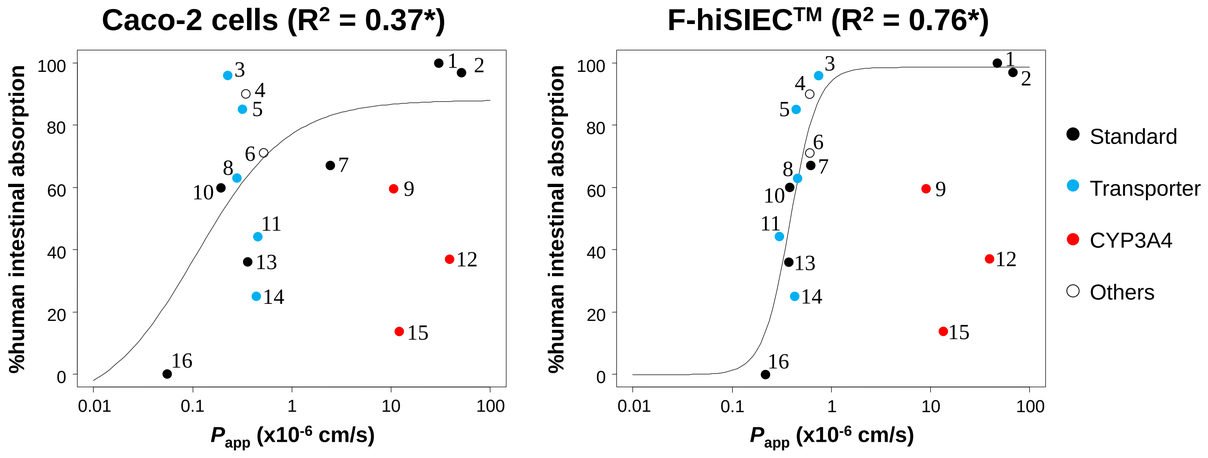
Morphology and barrier function



A) Cells were stained with villin 1. Transmission electron microscopy images of microvilli. B) Barrier function of cell sheet on cell culture insert by TEER.

The microvilli on the apical side of the cells and the barrier function were confirmed.

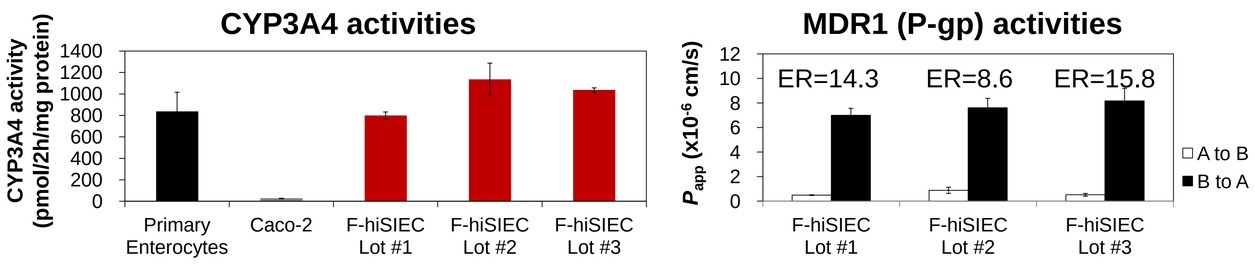
Drug permeability assay



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.

Activities of metabolizing enzyme and transporter



Each cells were incubated with 5 μM midazolam for 2h at 37 ℃. 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℃ for 60 min.

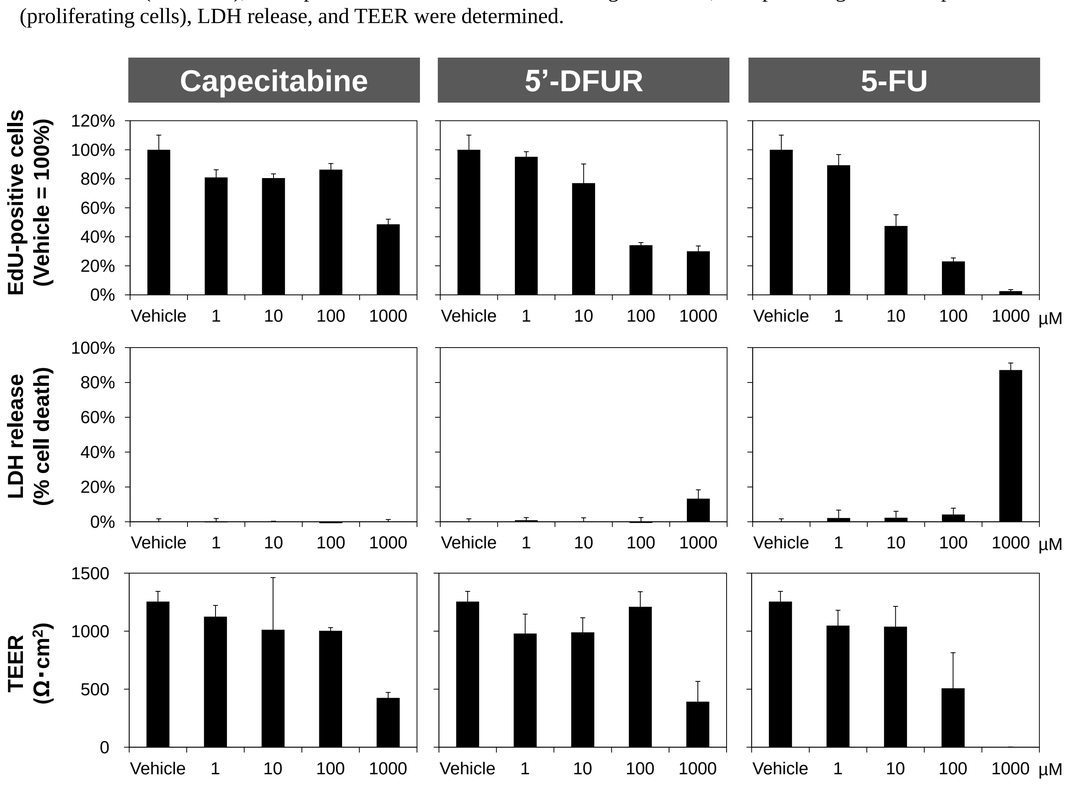
F-hiSIEC™ showed CYP3A4 activity comparable to that of human primary enterocytes. CYP3A4 activities and P-gp activities of F-hiSIEC™ could be experimented with good reproducibility.

GI toxicity model: Cytotoxic drugs

5-FU and its prodrugs

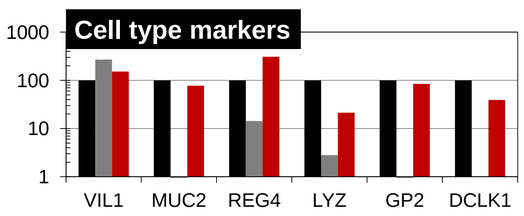
Capecitabine (prodrug) → (carboxylesterase) → 5'-DFCR → (cytidine deaminase) → 5'-DFUR (prodrug) → (thymidine phosphorylase) → 5-FU (cytotoxic)

The cell sheets of F-hiSIEC™ were treated with equivalent concentrations (1-1000 μM) of 5-FU, 5'-deoxy-5-fluorouridine (5'-DFUR), or capecitabine. At 72 hours after drug treatment, the percentage of EdU-positive cells (proliferating cells), LDH release, and TEER were determined.



The number of proliferating cells and barrier function were decreased in the 5-FU-treated group. Similar changes were also noted in the 5'-DFUR-treated and capecitabine-treated group, but those were less effective.

GI toxicity model: Gamma secretase inhibitors



F-hiSIEC™ contained goblet cells (MUC2), endocrine cells (REG4), Paneth cells (LYZ), M cells (GP2), and tuft cells (DCLK1), which constitute the small intestinal epithelium.

Notch regulation of intestinal differentiation

The cell sheets of F-hiSIEC™ were treated at concentrations of 0.1-10 μM gamma secretase inhibitors with Notch inhibitory activity (LY411575 and semagacestat) or gamma secretase modulators without Notch inhibitory activity (Tarenflurbil) during the differentiation process. Eight days after the treatment, the expression levels of MUC2 (a marker of goblet cells), ALPI and SI (markers of enterocytes) were determined by real-time PCR.

In the LY411575 or semagacestat-treated groups, there were dose dependent an increase of goblet cell marker and decreases of enterocyte markers, while no marked changes were observed in the tarenflurbil-treated group.

Conclusion: 1) F-hiSIEC™ showed similar characteristics to those of *in vivo* human small intestinal cells and can be used for predicting intestinal pharmacokinetics.
2) *In vitro* systems using F-hiSIEC™ can be a useful model for evaluating GI toxic agents and effects of drug candidates on differentiation of intestinal epithelium.

本発表は、2021 SOT Annual Meetingで発表した内容を含む。