JSSX Young Investigator's Award
(November 30, 2017)

ヒトiPS細胞由来組織細胞の作製と薬物動態試験への応用に関する研究

Generation of human iPS cell-derived tissue cells and application for pharmacokinetic studies

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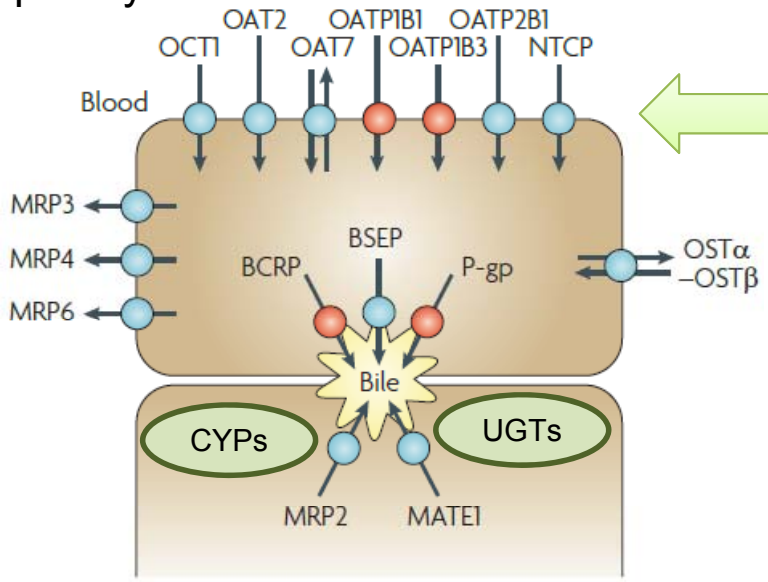
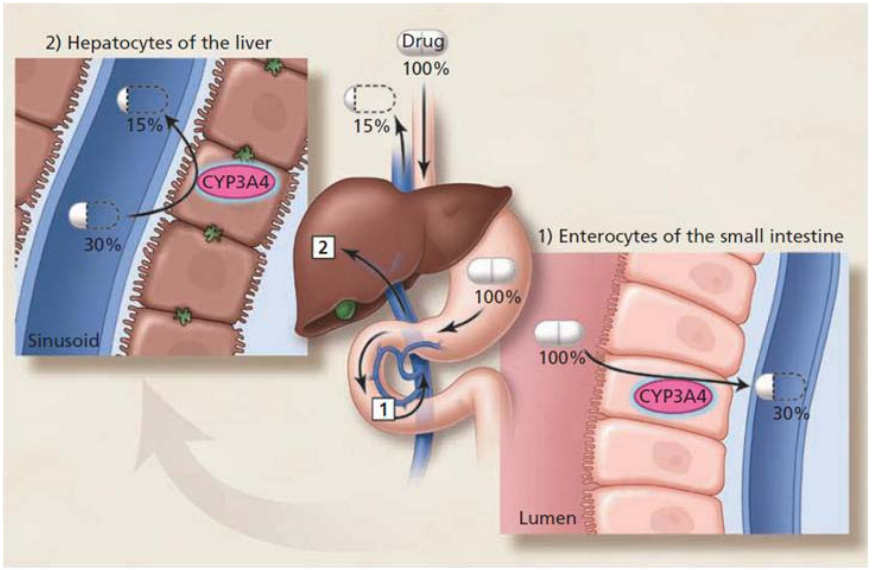
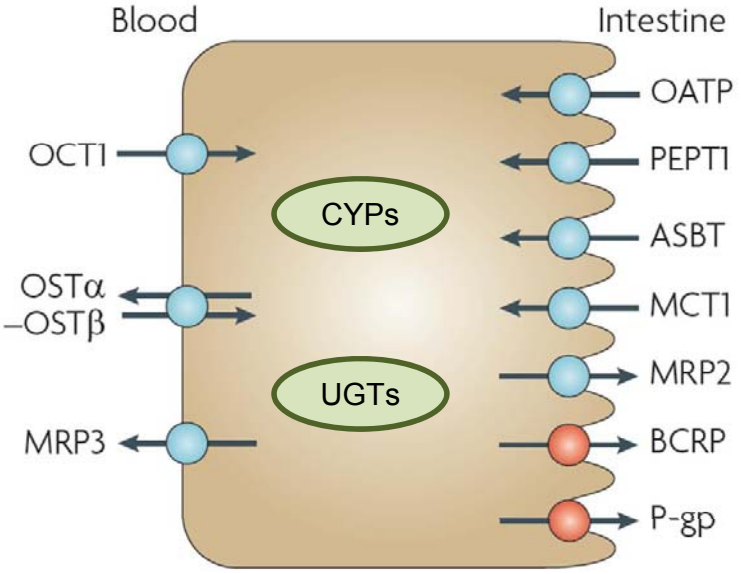
Importance of the intestine and liver in first-pass effects

Hepatocytes





Enterocytes



- Most of drugs are orally administrated.
- Many drug transporters and drug-metabolizing enzymes are expressed in the enterocytes and hepatocytes.
- The intestine and liver influence effects and/or side-effects of orally administrated drugs.

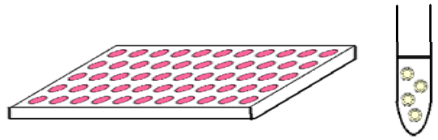
(*CMAJ*, **185**, 309, 2013; *Nat Rev Drug Discov*, **9**, 215, 2010)

Evaluation of pharmacokinetics and the issues

In vivo (animals)

- It is difficult to extrapolate the animal data to humans (species differences)

In vitro (cell lines, microsomes…)



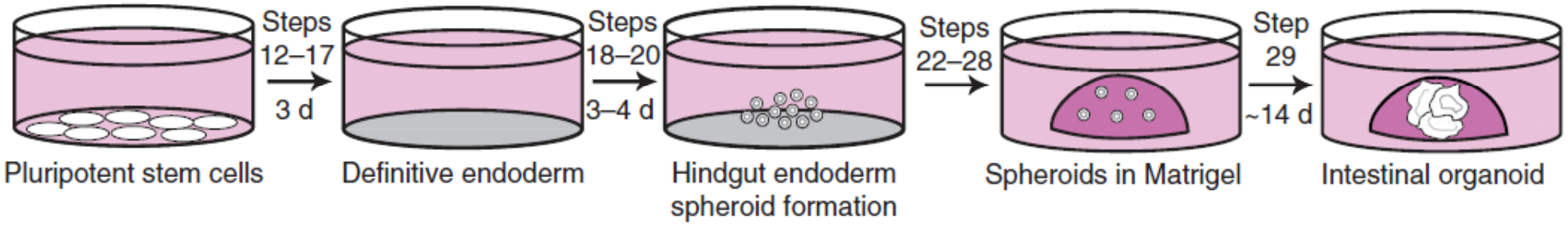
- Cell lines are ・・・
  - human carcinoma
  - difference of expression of drug transporters and drug-metabolizing enzymes compared to human tissues
- Primary hepatocytes have lot-to-lot variation.
- Drug absorption and metabolism are analyzed by diverse *in vitro* systems.
- It is almost impossible to obtain primary human enterocytes for pharmacokinetic studies.

【Purpose】Generation of enterocytes and hepatocytes from human iPS cells for developing novel pharmacokinetic evaluation systems

Differentiation human iPS cells to enterocytes

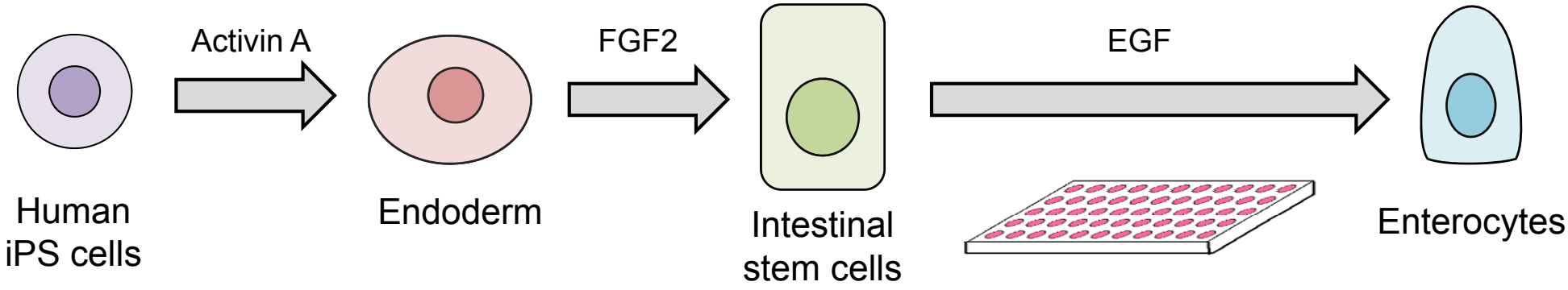
▪Differentiation of human iPS cells to organoids

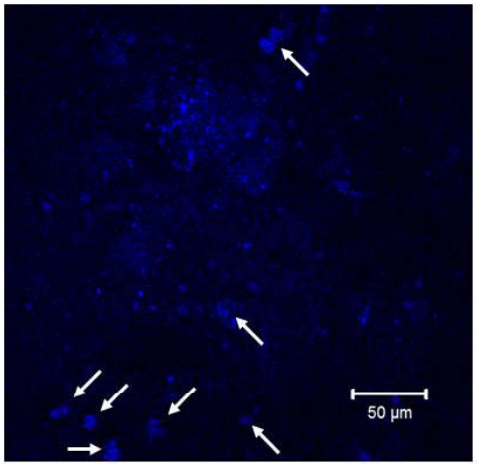
(*Nature, 470*, **105**, 2011; *Nat Protoc*, **6**, 1920, 2011)



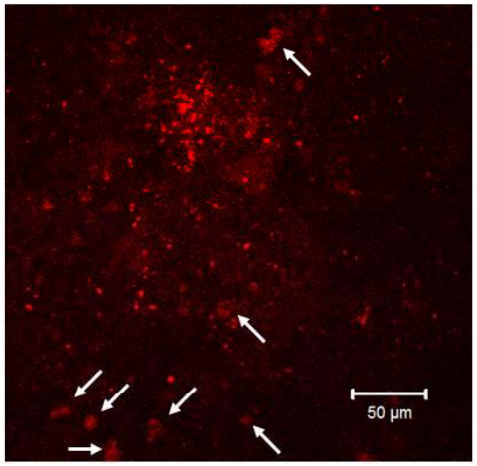
- ✓ Intestinal differentiation through definitive endoderm and hindgut by mimicking intestinal development
- ✓ Efficient differentiation to enterocytes
- ✓ 2D culture to predict intestinal drug absorption
- ✓ Pharmacokinetic functions (drug-metabolizing enzymes, drug transporters)

Differentiation human iPS cells to enterocytes

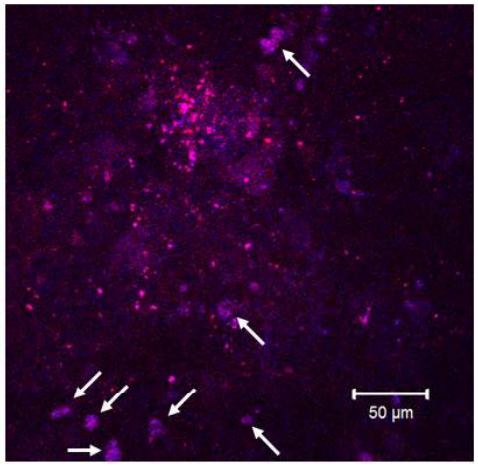




β-Ala-Lys-AMCA
（dipeptide）



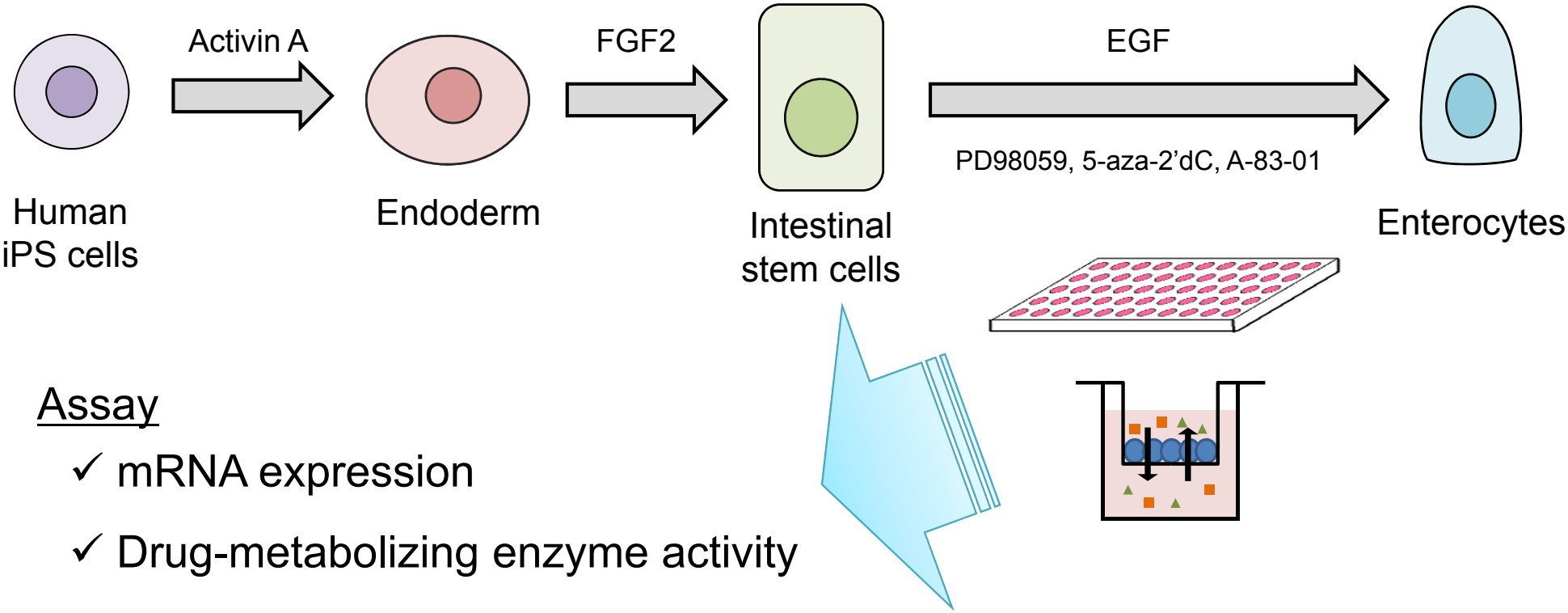
Sucrase–isomaltase



β-Ala-Lys-AMCA/
Sucrase–isomaltase

(*Drug Metab Pharmacokinet*, **29**, 44, 2014)

Differentiation human iPS cells to enterocytes

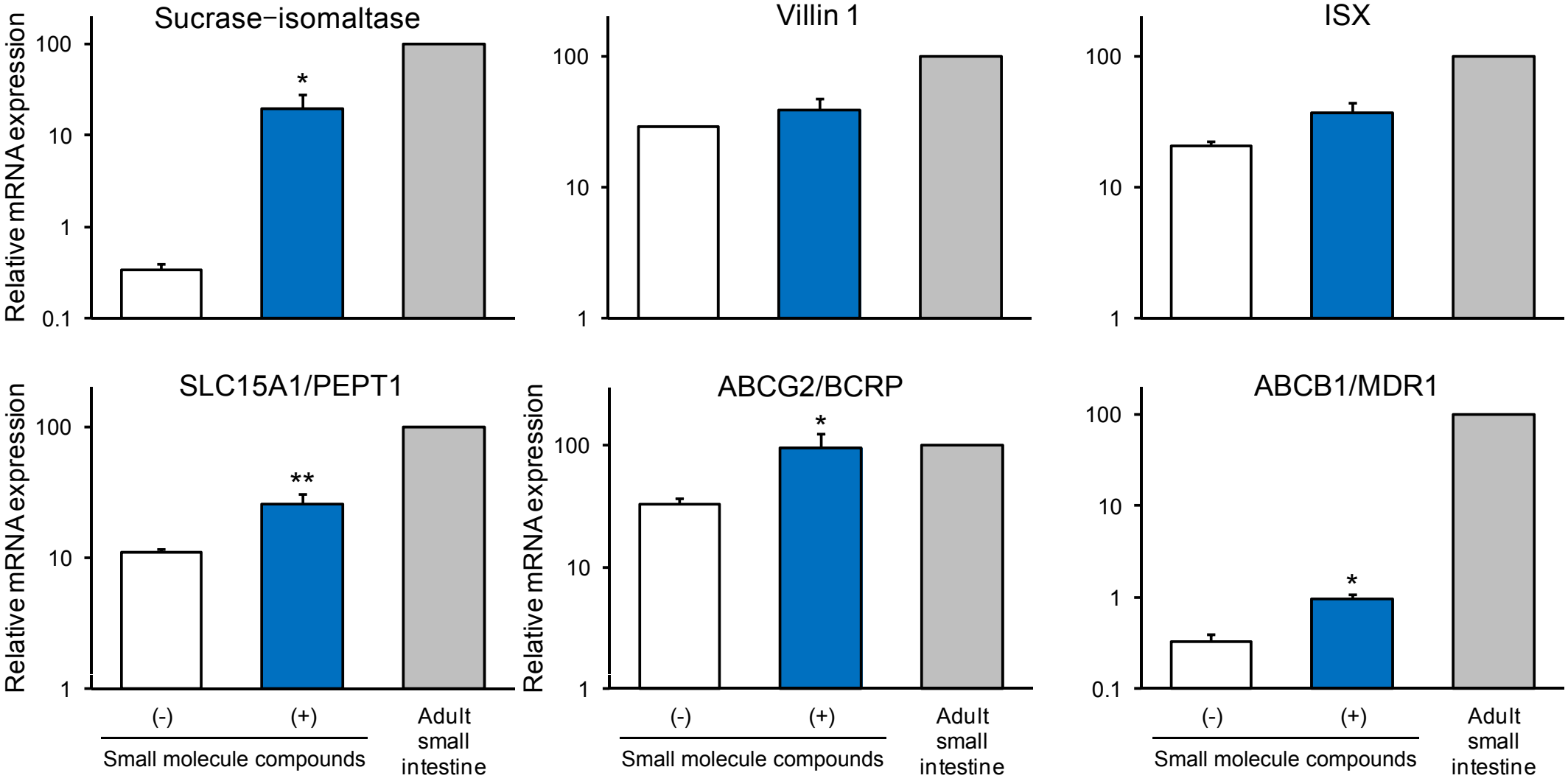


Assay

- ✓ mRNA expression
- ✓ Drug-metabolizing enzyme activity
- ✓ CYP3A4 inducibility
- ✓ Transport activity

(*Drug Metab Dispos*, **43**, 603, 2015)

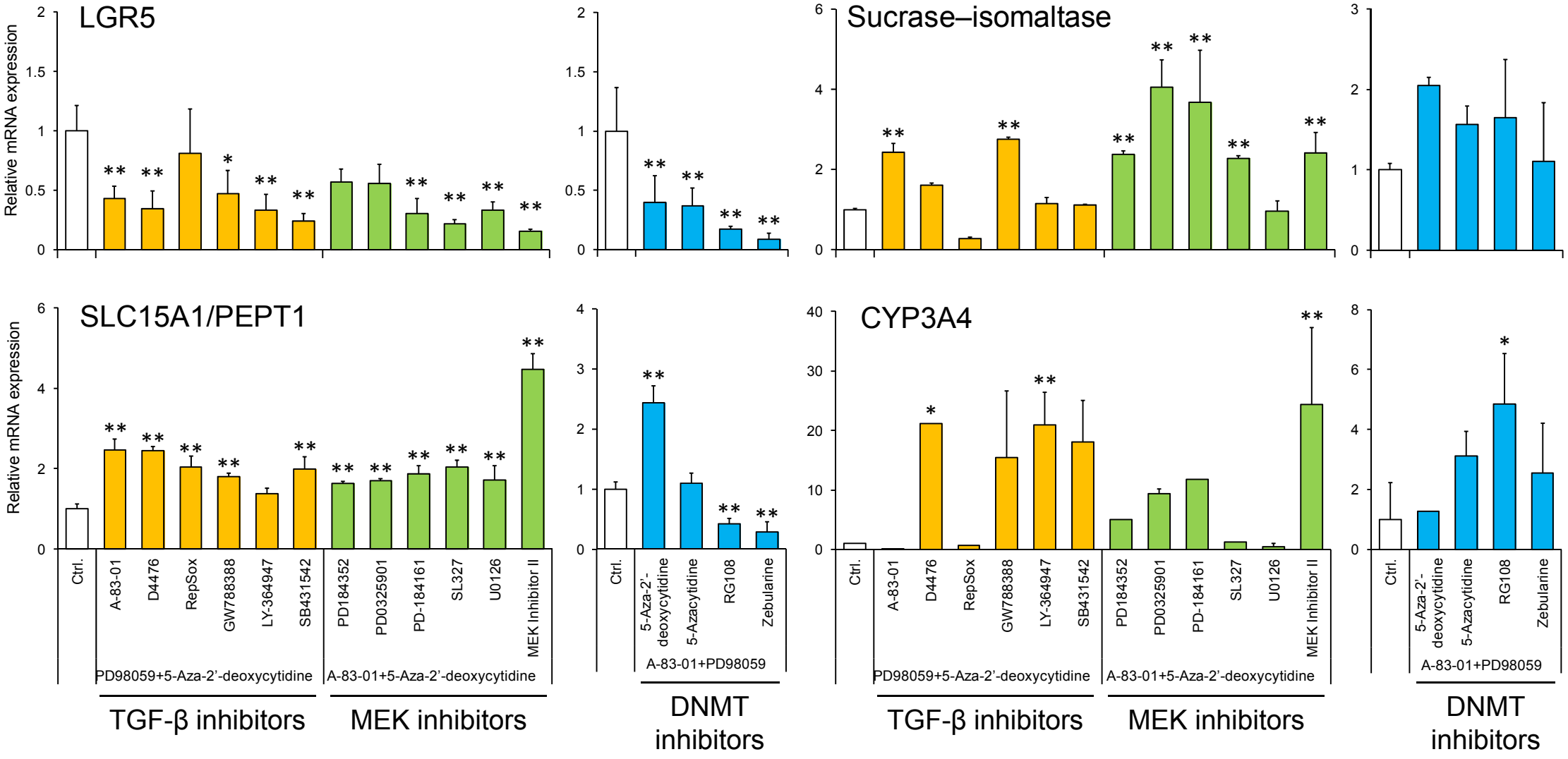
mRNA expression of intestinal markers and drug transporters



The expression of the intestinal markers and drug transporters were increased.

*p < 0.05; **p < 0.01. (*Drug Metab Dispos*, **43**, 603, 2015)

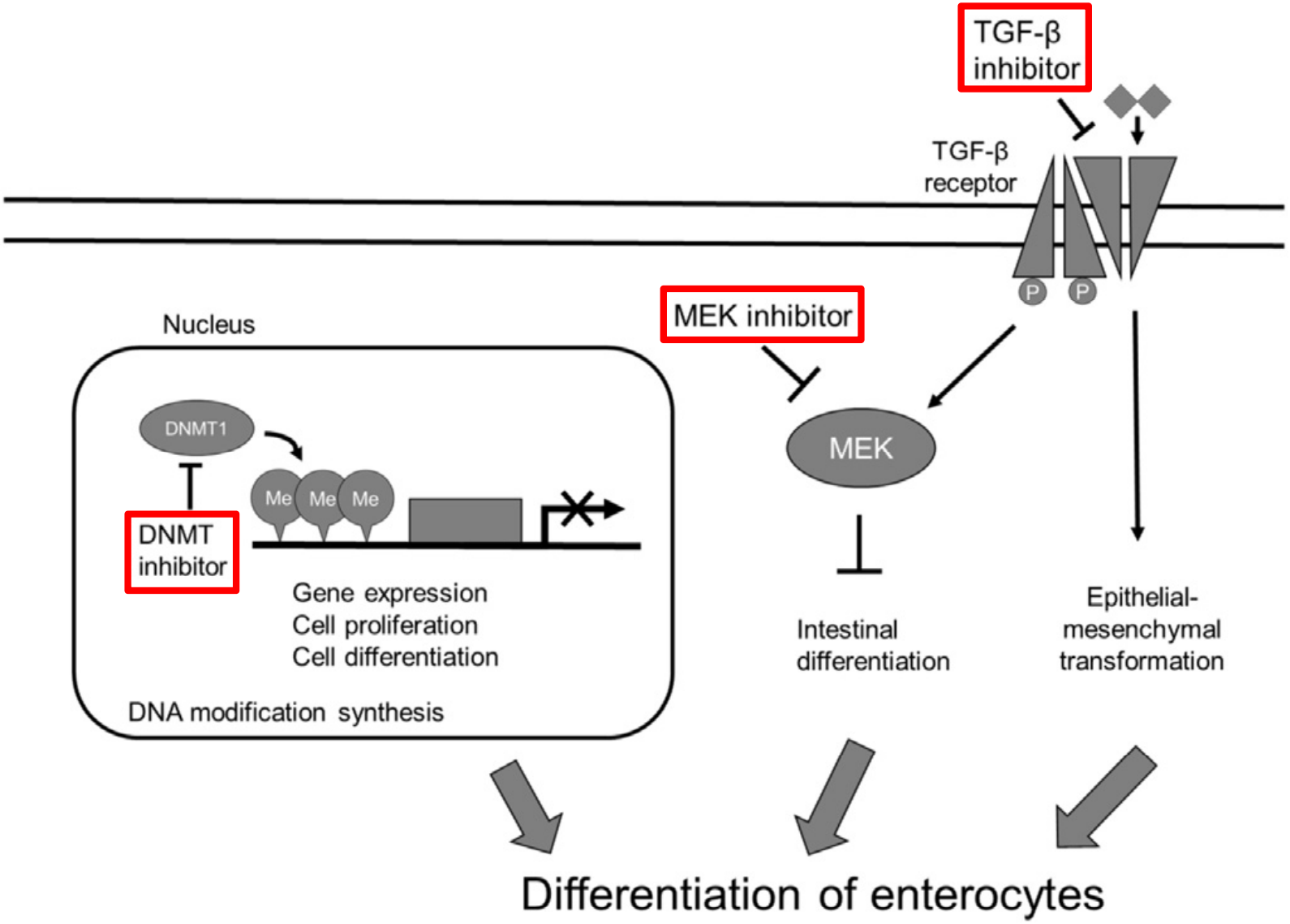
Effects of MEK, DNMT, and TGF-β inhibitors for the intestinal differentiation



*$p < 0.05$, **$p < 0.01$.

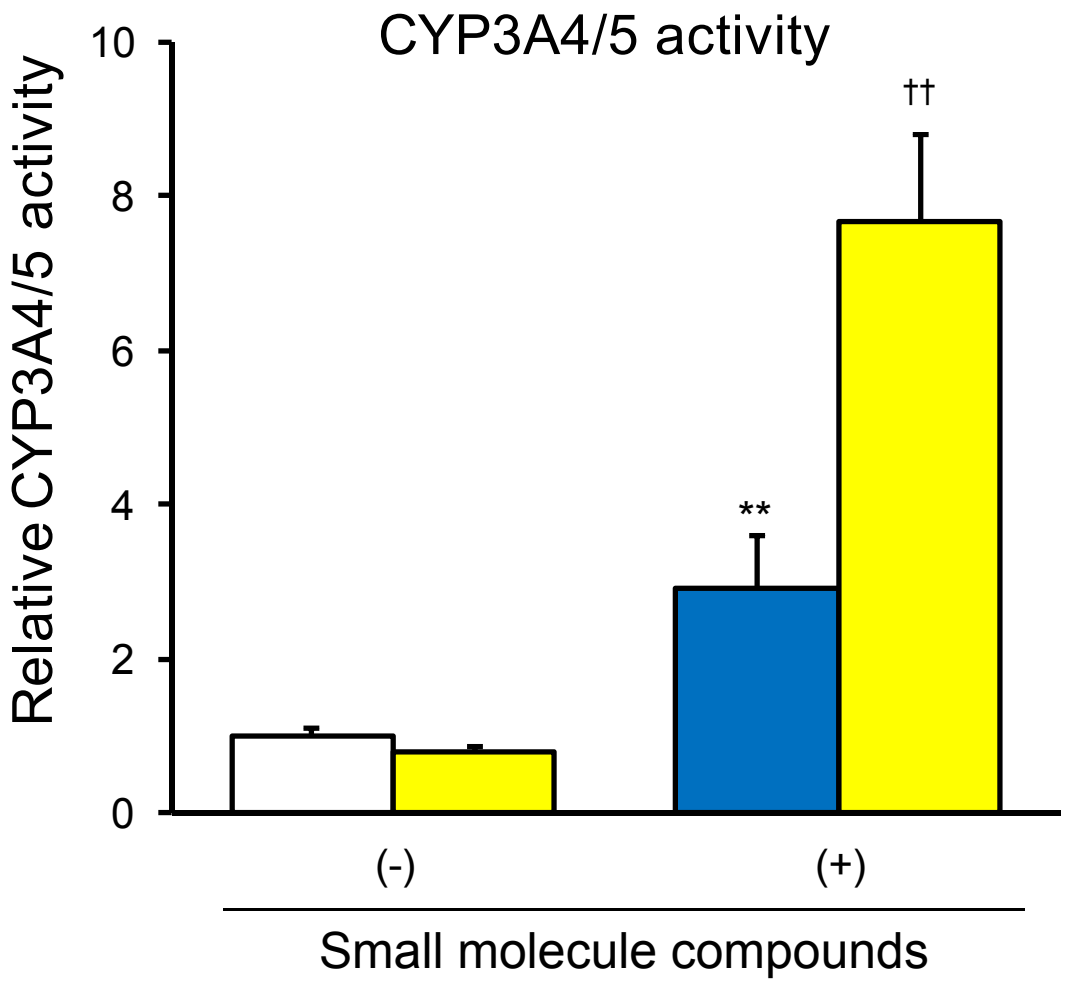
(*Drug Metab Pharmacokinet*, **31**, 193, 2016)

A scheme of the approach for systematic differentiation of human iPS cells to enterocytes



(*Drug Metab Pharmacokinet*, **31**, 193, 2016)

CYP3A4 inducibility in human iPS cell-derived enterocytes



CYP3A4 expression and CYP3A4/5 activity were induced.

□■ Vehicle
□ 1α,25-dihydroxyvitamin D_3

**$p < 0.01$ vs non-treatment group;
††$p < 0.01$ vs vehicle group.

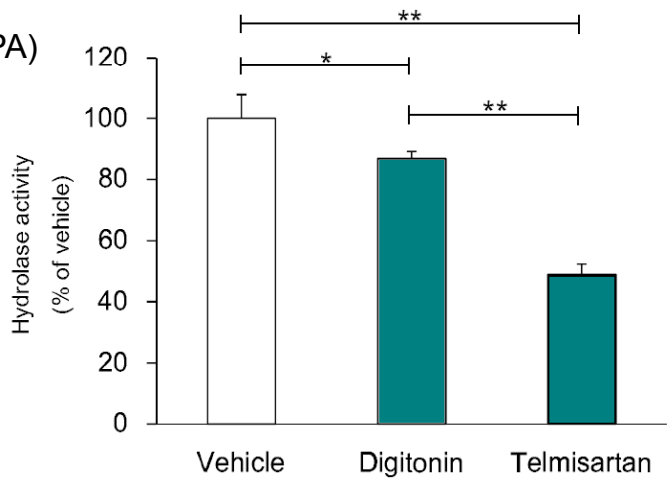
(*Drug Metab Dispos*, **43**, 603, 2015)

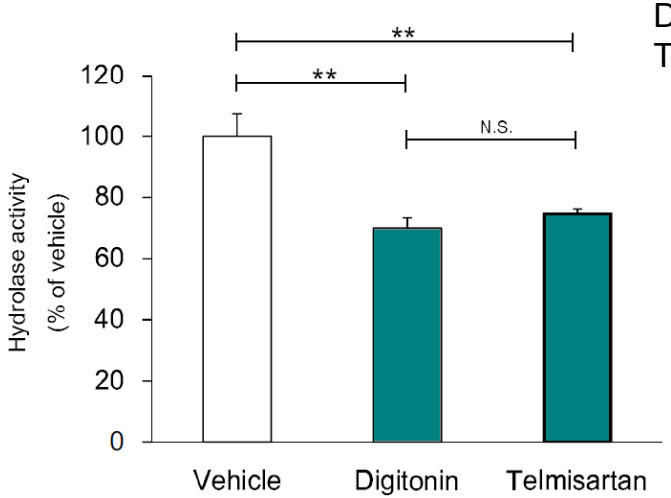
CES activity in human iPS cell-derived enterocytes

Human iPS cell-derived enterocytes

Caco-2 cells

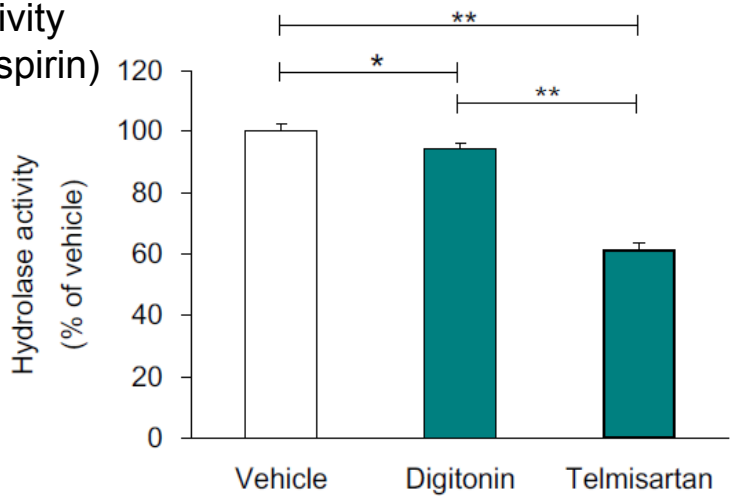
CES activity
(Substrate: PNPA)

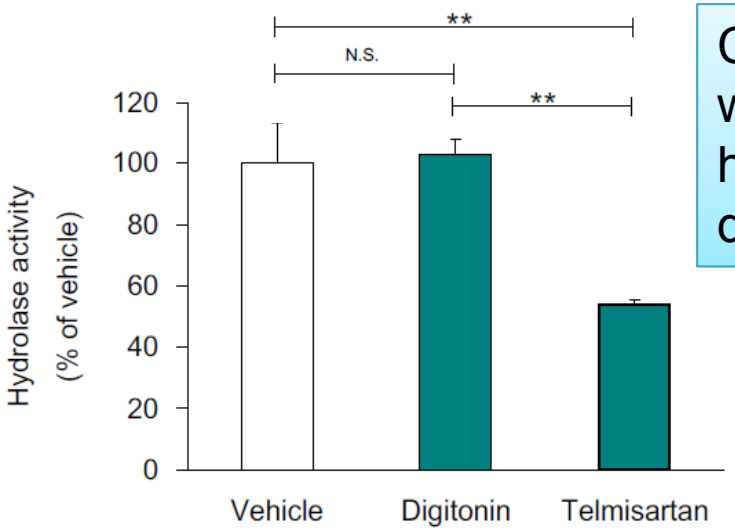




Digitonin: CES1A inhibitor
Telmisartan: CES2A1 inhibitor

CES2A1 activity
(Substrate: aspirin)

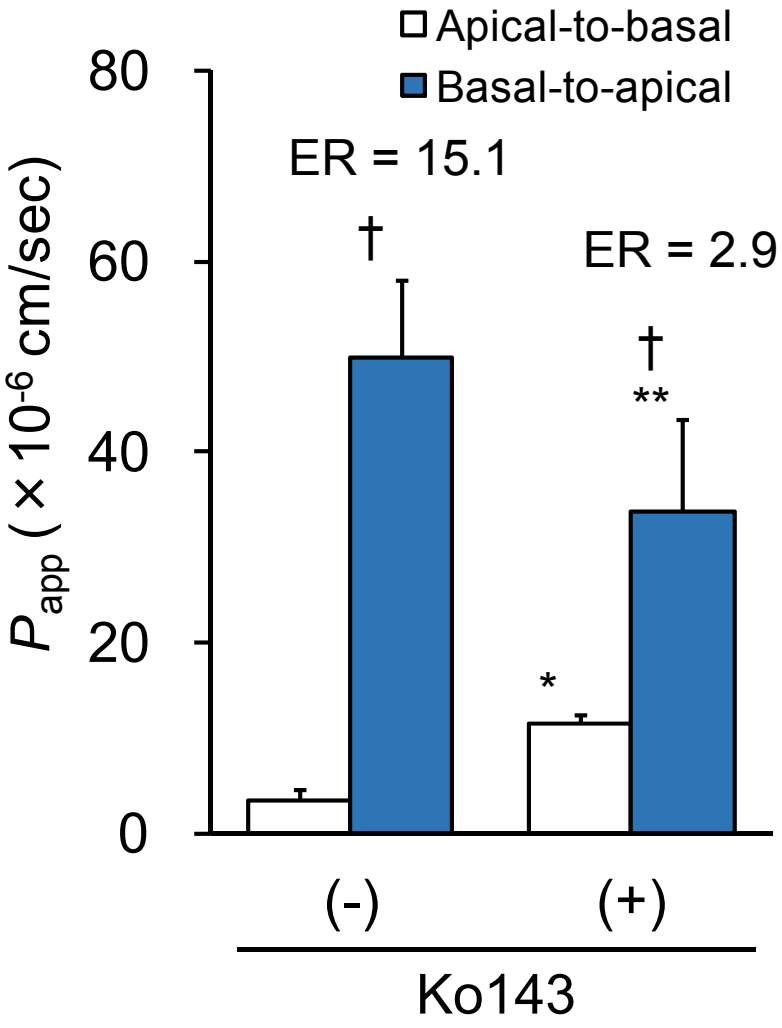




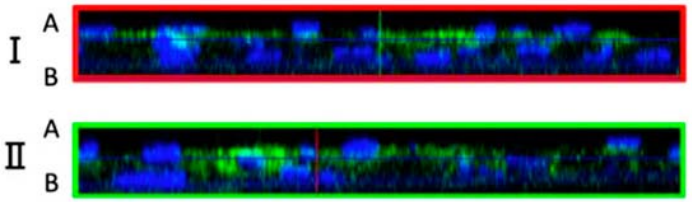
CES2A metabolism was significant in human iPS cell-derived enterocytes.

(*Biochem Biophys Res Commun*, **486**, 143, 2017)

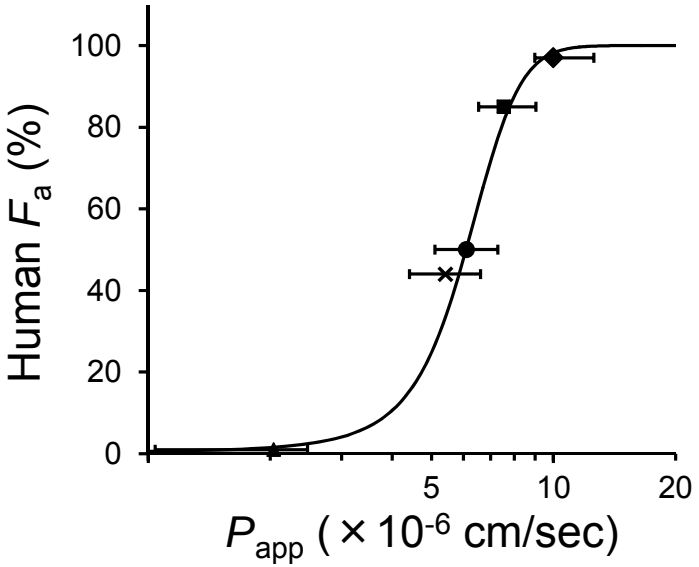
Bidirectional permeability mediated by BCRP

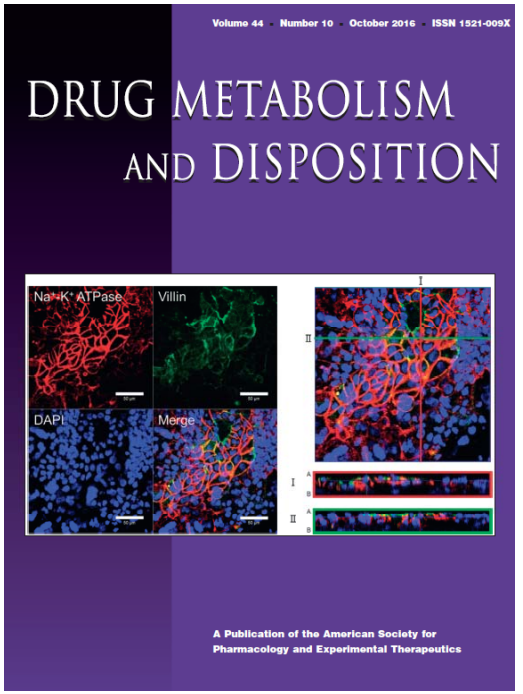


*p < 0.05, **p < 0.01 vs P_{app} values without Ko143; †p < 0.01 vs apical-to-basal P_{app} values.



A, apical side; B, basal side.
Green, BCRP; blue, DAPI.



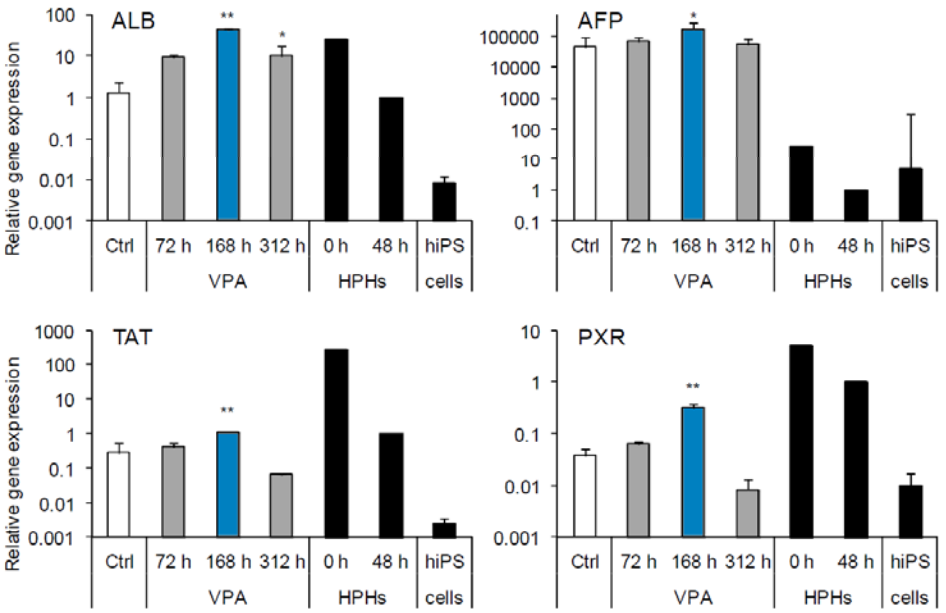


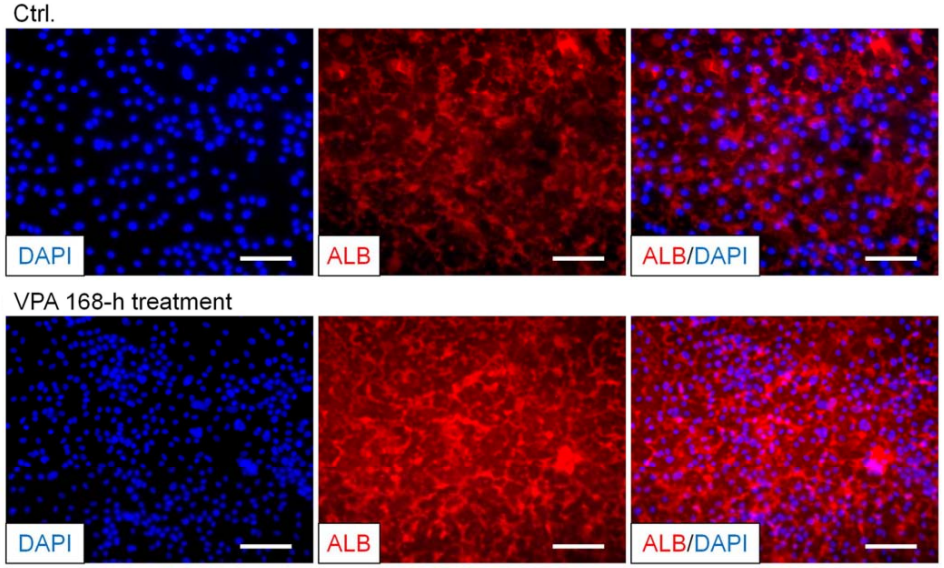
◆ Antipyrine
■ Metoprolol
● Atenolol
× Sulpiride
▲ PEG4000

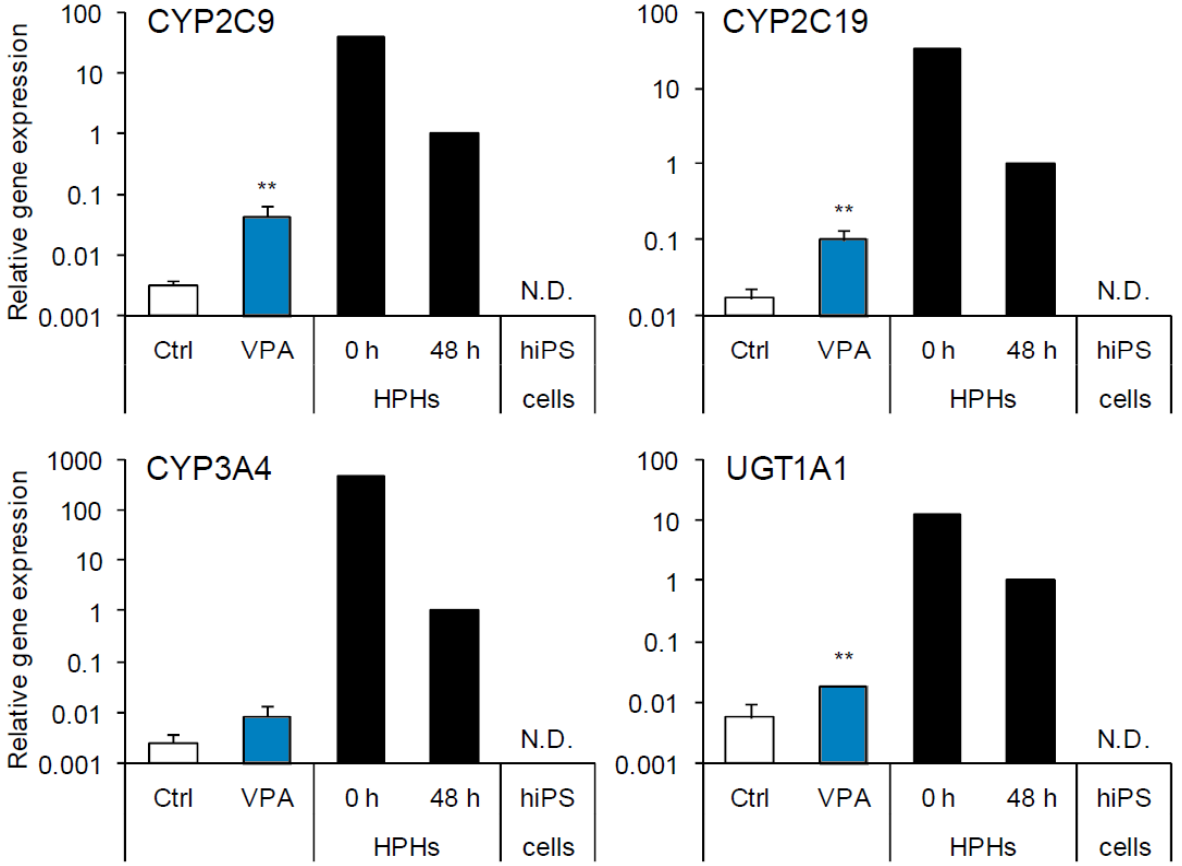
P < 0.01, R = 0.99

(*Drug Metab Dispos*, **44**, 1662, 2016)

Valproic acid promotes hepatic differentiation of human iPS cells



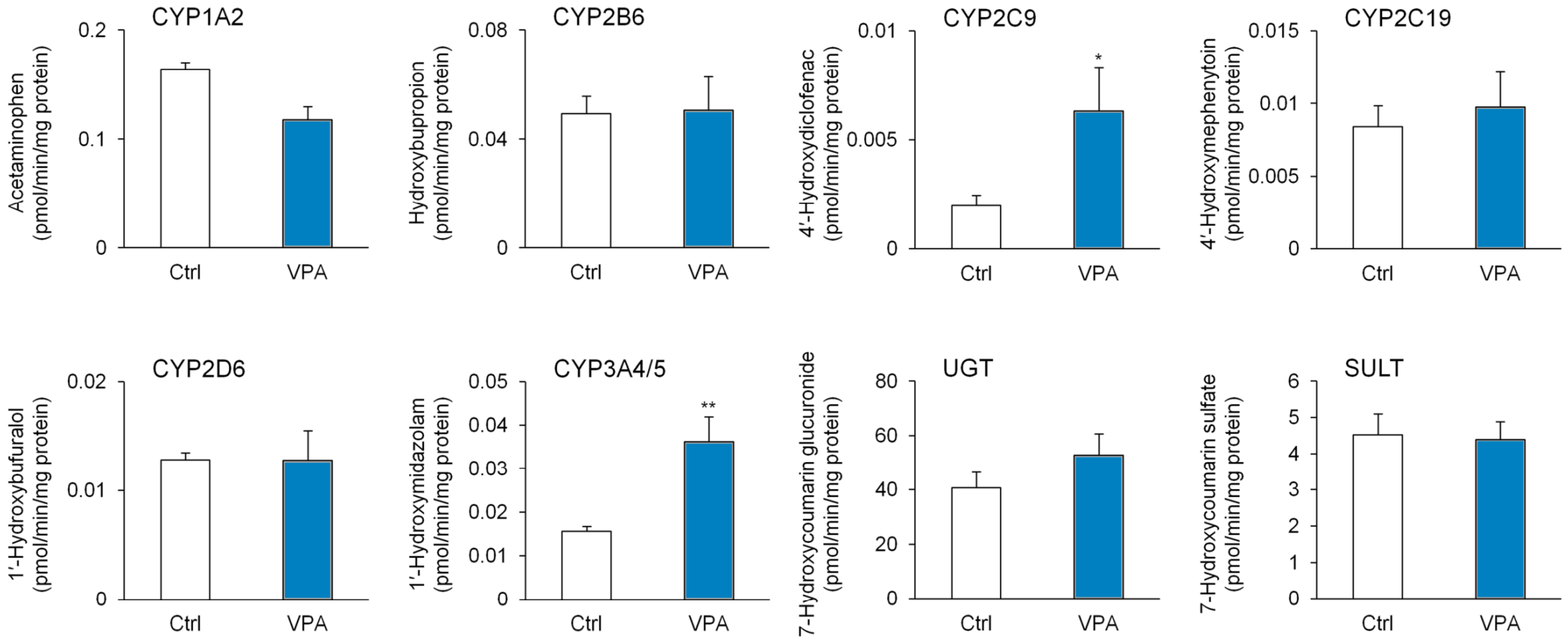




- The expression of hepatic markers and drug-metabolizing enzymes were increased by the treatment of valproic acid.

(*PLoS One*, **9**, e104010, 2014)

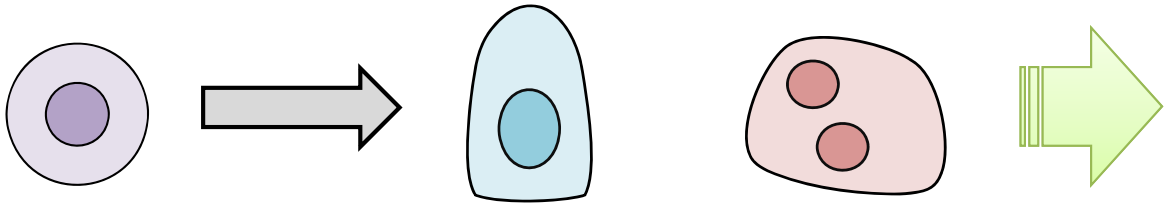
Drug-metabolizing activities in the human iPS cell-derived hepatocytes



(*PLoS One*, **9**, e104010, 2014)

Summary

- We found novel small molecule compounds to promote the differentiation and established the differentiation methods of human iPS cells to enterocytes and hepatocytes.
- Human iPS cell-derived enterocytes had following characteristics:
  - The expression of drug transporters and drug-metabolizing enzymes
  - Drug-metabolizing enzyme activities (CYPs, UGT, SULT, and CES)
  - CYP3A4 inducibility (VDR, PXR)
  - Uptake and efflux transporter activities (PEPT1, OATP, BCRP, P-gp)
  - Apical/basal polarity
- Human iPS cell-derived hepatocytes also had drug-metabolizing enzyme activities and CYP inducibility.



Comprehensive evaluable models in the prediction of human intestinal and hepatic pharmacokinetics

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