Development of Highly Sensitive and Specific *in vitro* Renal Solute Carrier (SLC) Uptake Cell Models Using Genetically Modified Human Renal Proximal Tubule Epithelial Cells for Renal Drug Transporter Interaction Studies

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Abstract

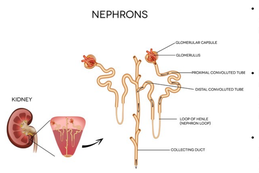
The disposition and clearance of drugs by the kidney relies largely on a well-characterized subset of membrane transport pumps collectively known as solute carrier (SLC) proteins. Among the SLC family proteins, OAT1, OCT2, and OAT3 are considered most important transporters in kidney tissue, and are recommended by the FDA, ITC, and EMA as targets for drug-drug interaction studies. Therefore, there is a large demand for *in vitro* kidney transporter models that have normal human kidney origin, functioning transporters, clinical predictability, and consistent data output for drug interaction studies. Unfortunately, primary renal epithelial cells lose OAT1, OCT2, and OAT3 transporter expression quickly in culture. Transiently expressing these transporters in primary renal epithelial cells yields large variations between experimental models making data hard to interpret. Current cell line-based models are available using MDCK, CHO, U2OS, or other lines which either do not have human kidney tissue origin or are themselves cancer lines, significantly compromising clinical predictability. In our study, we have generated kidney transporter cell models using well-characterized hTERT-immortalized primary Renal Proximal Tubule Epithelial Cells that stably overexpress either the OAT1, OCT2, or OAT3 gene. After confirming the SLC mRNA expression for each gene by RT-PCR, we performed immunostaining that showed that OAT1, OCT2, and OAT3 are correctly trafficked to the plasma membrane. Notably, those clones show typical epithelial morphology, functionality, and expression of the appropriate epithelial and kidney tissue specific markers. Most importantly, we verified that the overexpressed transporters have normal transport activities using 6-CF and EAM-1 uptake assays in a high throughput format. We also show that uptake of these compounds are blocked in a dose dependent manner by well-known SLC inhibitors, indicating that the overexpressed kidney transporters are functioning as expected. Overall, our data demonstrates that these modified renal epithelial cell lines maintain kidney transporter expression over time, and provide physiologically relevant high sensitive and specific data regarding human kidney transporter functions. These models are consistent and reliable, and can be very valuable tools for high throughput kidney drug toxicity screening to test the effects of exogenous compounds on renal membrane transporter function.

Introduction

- Transport proteins play very important roles in the absorption, distribution, and elimination of a wide variety of drugs from the kidney.
- OAT1 (SLC22A6), OCT2 (SLC22A2), and OAT3 (SLC22A8) are the most important transporters in kidney tissue and are recommended for drug interaction studies by the ITC, FDA, and EMA.
- Primary RPTEC cells lose SLC transporter expression over time in culture. Transiently expressing these transporters in primary RPTEC cells yields large variations between production lots making data hard to interpret.
- Drug transporter interactions always happen in the kidney micro-environment, which means cell origination is very important in determining the accurate predictability for an *in vitro* models
- Current cell line-based models of kidney transporter function are available using MDCKII, CHO, U2OS, or other lines, which either do not have a human kidney tissue origination or are themselves cancer lines, significantly compromising their clinical predictability. In this study, we generated renal SLC uptake models using true kidney proximal tube epithelial cells: RPTEC/TERT1.

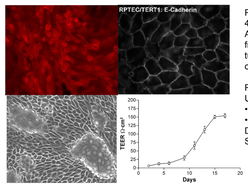
Results

1. Background and cell lines generation



https://ghr.nlm.nih.gov/condition/renal-hypouricemia

- The kidney is one of the major target organs for drug-induced toxicity; due to the large functional reserve of the kidney, nephrotoxic effects often become obvious only after regulatory approval
- Nephrotoxic potential is often underestimated when new drugs are available, which leads again to clinical complications, as in the case of COX2 inhibitors
- The kidney renal proximal tubule (PT) are a major target for drug-induced toxicity due to their roles in glomerular filtrate concentration and the transport of drugs and organic compounds.
- The transporter proteins (ABC and SLC) in the proximal tubule epithelial cells play important roles for drug: Absorption, Distribution and Elimination



RPTEC/TERT1 (ATCC® CRL-4031™)
An epithelial cell line isolated from human renal proximal tubes immortalized by hTERT* only

RPTEC/TERT1 exhibit:
Uniform expression of:
- E-cadherin
- CD13 (aminopeptidase N)

Dome-like structures
Stabilized TEER

*hTERT refers to human Telomerase reverse transcriptase, an enzyme which catalyzes the synthesis of telomeres at the end of Chromosomes; in normal human primary cells telomeres shorten after each division, after a certain number of division, the telomere will be completely lost and the cells will go to senescence. One way to bypass replicative senescence is to overexpress hTERT. Therefore, hTERT is a naturally existing component in the cell, which has the minimum impact to the primary cells while bypassing replicative senescence.

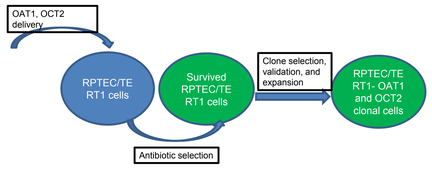


Figure 1. Generation of stably overexpressing OAT1, OCT2, and OAT3 RPTEC/TERT1 cell lines. RPTEC/TERT1 cells were transduced with retrovirus (SV40 viral promotor) containing either OAT1, OCT2, and OAT3. After 24 hours, the cells were selected with antibiotic for 7 days. The surviving cells were subjected to clonal isolation and expanded. The isolated clones were validated for clonality, kidney transporter expression, and kidney transporter activity.

2. Correct Expression and Membrane Localization of OAT1, OCT2 and OAT3 in RPTEC/TERT1 Cells

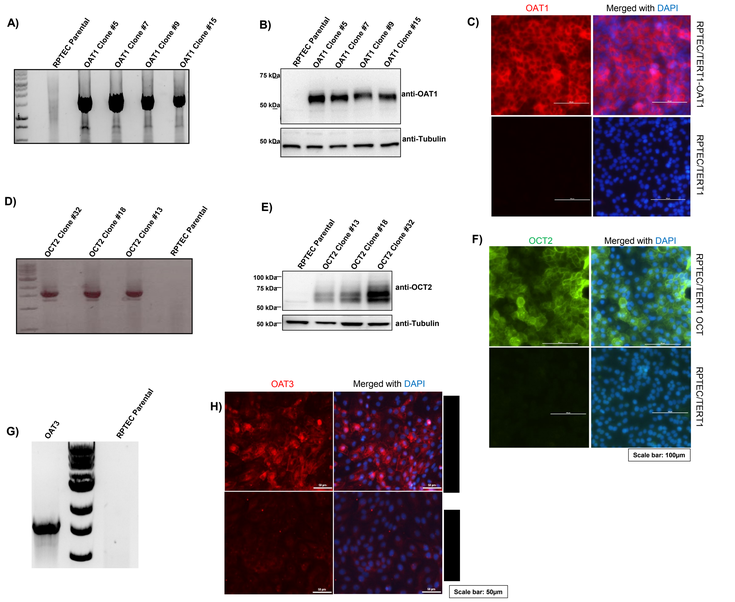


Figure 2. Molecular characterization of the RPTEC SLC transporter cells. A) RT-PCR demonstrates the presence of OAT1 mRNA in RPTEC/TERT1 OAT1 cells. **B)** Immunoblot demonstrates OAT1 protein expression levels in RPTEC/TERT1 parental and OAT1 cells. **C)** Immunofluorecene/Immunocytochemistry (IF/ICC) demonstrates OAT1 expression and localization. **D)** RT-PCR demonstrates the presence of OCT2 mRNA in RPTEC/TERT1 OCT2 cells. **E)** Immunoblot demonstrates OCT2 protein expression levels RPTEC/TERT1 parental and OCT2 cells. **F)** IF/ICC demonstrates OCT2 expression and localization. **G)** RT-PCR demonstrates the presence of OAT3 mRNA in RPTEC/TERT1 OAT1 cells and **H)** IF/ICC demonstrates OAT3 expression and localization.

3. Modified RPTEC/TERT1 SLC Transporter Cell Lines Retain the Renal Epithelial Cell Characteristics of their Parental Counterpart

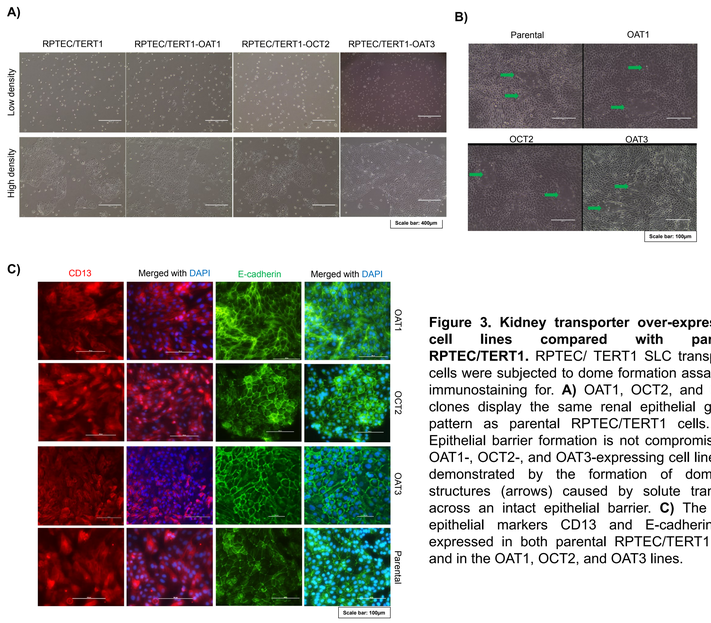
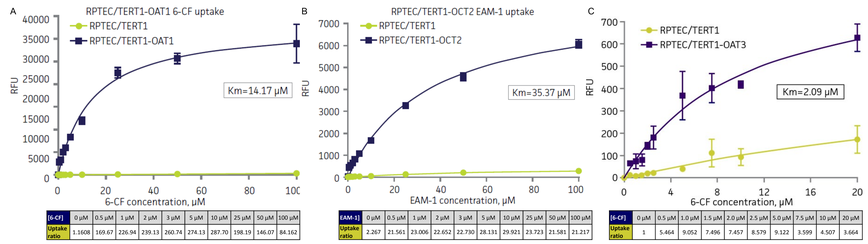


Figure 3. Kidney transporter over-expressing cell lines compared with parental RPTEC/TERT1. RPTEC/ TERT1 SLC transporter cells were subjected to dome formation assay and immunostaining for. **A)** OAT1, OCT2, and OAT3 clones display the same renal epithelial growth pattern as parental RPTEC/TERT1 cells. **B)** Epithelial barrier formation is not compromised in OAT1-, OCT2-, and OAT3-expressing cell lines, as demonstrated by the formation of dome-like structures (arrows) caused by solute transport across an intact epithelial barrier. **C)** The renal epithelial markers CD13 and E-cadherin are expressed in both parental RPTEC/TERT1 cells and in the OAT1, OCT2, and OAT3 lines.

4. RPTEC/TERT1-OAT1, -OCT2 and -OAT3 Display High Uptake Sensitivities



[6-CF]	0 μM	0.5 μM	1 μM	2 μM	3 μM	5 μM	10 μM	25 μM	50 μM	100 μM
Uptake ratio	1.1608	169.67	226.94	239.13	260.74	274.13	287.70	198.19	146.07	84.162

[EAM-1]	0 μM	0.5 μM	1 μM	2 μM	3 μM	5 μM	10 μM	25 μM	50 μM	100 μM
Uptake ratio	2.267	21.561	23.006	22.652	22.730	28.131	29.921	23.723	21.581	21.217

[6-CF]	0 μM	0.5 μM	1.0 μM	1.5 μM	2.0 μM	2.5 μM	5.0 μM	7.5 μM	10 μM	20 μM
Uptake ratio	1	5.464	9.052	7.496	7.457	8.579	9.122	3.599	4.507	3.644

Figure 4. Drug Kinetic Profiles of RPTEC/TERT1 OAT1 and OCT2 Transporter Cells. A) Solute uptake activity of RPTEC/TERT1 OAT1 cells was assessed using 6-CF as substrate. As expected, uptake increases with increasing 6-CF concentration in OAT1-expressing cells but not in parental RPTEC/TERT1 cells (n=3), indicating that the observed transport is due to OAT1 expression. **B)** Solute uptake activity of RPTEC/TERT1 OCT2 cells was assessed using EAM-1 as substrate. As expected, uptake increases with increasing amounts of EAM-1 in OCT2-expressing cells but not in parental RPTEC/TERT1 cells (n=3), indicating that the observed solute transport is due to OCT2 expression. **C)** Solute uptake activity of RPTEC/TERT1 OAT3 cells was assessed using 6-CF as substrate. As expected, uptake increases with increasing 6-CF concentration in OAT1-expressing cells but not in parental RPTEC/TERT1 cells (n=3), indicating that the observed transport is due to OAT3 expression(n=3)

5. RPTEC/TERT1-OAT1, -OCT2 and -OAT3 Transporter Cell Lines Show Specific Uptake Activities Indicated by Responses to Well-known SLC Inhibitors

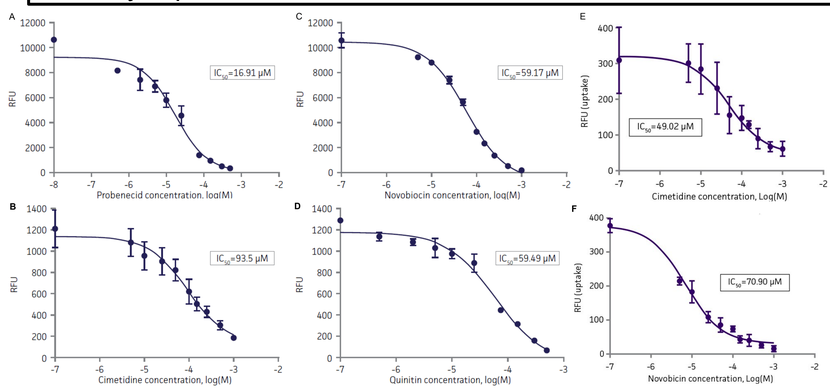


Figure 5. Transport inhibition kinetics of RPTEC/TERT1 OAT1-, OCT2-, and OAT3-expressing cell lines. A) and **B)** OAT1- expressing cells were exposed to increasing concentrations of the known OAT1 inhibitors probenecid and novobiocin while 6-CF concentration and uptake time were held constant at 3 μM and 20 minutes, respectively. **C)** and **D)** OCT2 expressing cells were exposed to increasing concentrations of the known OCT2 inhibitors cimetidine and quinitin while EAM-1 concentration and uptake time were held constant as above. The resulting inhibition curves indicate that OAT1 and OCT2 have physiologically relevant transport activity when overexpressed in RPTEC/TERT1 cells; **E)** and **F)** OAT3-expressing cells were exposed to increasing concentrations of the known OAT3 inhibitors cimetidine and novobiocin while 6-CF concentration and uptake time were held constant at 5 μM and 20 minutes, respectively. (n=3).

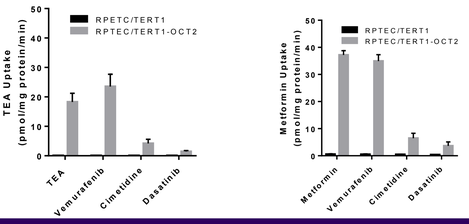


Figure 6. Independent third-party confirmation of RPTEC/TERT1 OCT2 uptake sensitivity and specificity using two known OCT2 substrates (TEA and Metformin) and two known OCT2 inhibitors (Cimetidine and Dasatinib) as well as a negative control (Vemurafenib): (data generated in Dr. Allison Gibson's Lab, Ohio State University)

Summary

- We have generated and characterized cellular models for renal solute uptake by stably expressing OAT1, OCT2, and OAT3 in RPTEC/TERT1.
- OAT1, OCT2, and OAT3 overexpressing cell lines retain the relevant renal epithelial characteristics of the parental cell line.
- Stable transporter expression in RPTEC/TERT1 cells was confirmed by PCR, immunostaining, and immunoblot.
- All three model lines perform well in cellular uptake assays of known OAT1, OCT2, and OAT3 transport substrates, making them valuable tools for drug-drug interaction studies and/or clinical trials.
- Transport activity in both model cell lines is inhibited by known SLC inhibitors such as probenecid and cimetidine, further demonstrating their use as physiologically relevant models of kidney function.

References

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