hTERT-immortalized Kidney Models for Drug Screening and Toxicity Assays

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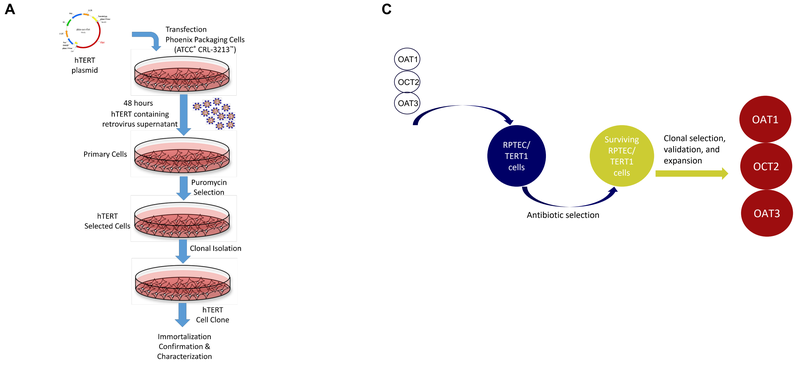
Abstract

Kidneys are the major organs in the body responsible for the elimination of many xenobiotics and prescription drugs; having relevant models for drug interaction and toxicity studies is a necessity. Primary cells and continuous cell lines have traditionally been used in these studies. We have generated human telomerase reverse transcriptase (hTERT) immortalized renal proximal tubule epithelial cells (hTERT-RPTEC) that can overcome the limitations of donor variability and senescence of primary cells, yet show key primary cell functionality. These cells have the expected expression of E-cadherin and CD31 and display typical dome formation morphology. One limitation of primary RPTEC is that the expression of key solute carrier (SLC) transporters OAT1, OCT2, and OAT3 is lost during culture and expansion of these cells. To overcome this loss of expression, we have stably overexpressed each of these carriers in the hTERT-RPTEC background. These cells maintain correct marker expression (both overexpressed SLC and endogenous RPTEC markers) and growth characteristics. Functional uptake studies using fluorescently labeled 6-carboxyfluorescein (6-CF) and ethanaminium iodide (EAM-1), and inhibition of these uptakes by novobiocin and cimetidine demonstrate the functionality of the overexpressed SLCs. These cells have also been used to study the mechanism of drug toxicity (*e.g.*, antiviral drugs tenofivir, tenofivir DF, cidofivir) using cell viability assays. In aggregate, these data demonstrate that the immortalized hTERT-RPTEC and the SLC-expressing stable variants (hTERT-RPTEC-OAT1, -OCT2 and -OAT3) are reliable kidney models that can be used for drug screening and toxicity assays.

Results

I. Generation of Immortalized RPTEC/TERT1 OAT1, OCT2, and OAT3 Kidney cells



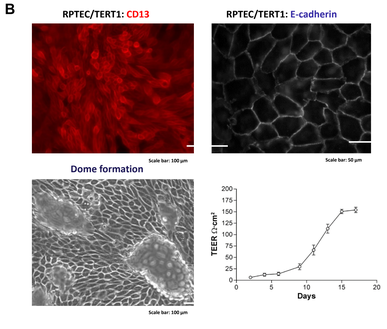


Figure 1. Generation of stably overexpressing OAT1, OCT2, or OAT3 RPTEC/TERT1 cell lines. (A) Principle of generating immortalized primary cells by using hTERT. The process involves the production of hTERT-containing virus supernatant that is subsequently used to infect target primary cells. These cells are grown under antibiotic selection pressure, and single cells are then isolated and characterized for functionality and immortalization. (B) RPTEC/TERT1 cells show many characteristics of primary RPTEC cells: expression of CD13 and E-cadherin (top panel), dome formation (bottom left), and transepithelial resistance (bottom right). (C) To generate the OAT1, OCT2, and OAT3 overexpressing cells, RPTEC/TERT1 cells were transduced with retrovirus containing either OAT1, OCT2, or OAT3 under the control of the SV40 viral promotor. The cells were selected with antibiotic for 7 days and surviving cells were subjected to clonal isolation and expanded. The isolated clones were validated for clonality, kidney transporter expression, and kidney transporter activity.

II. Marker Expression in Immortalized RPTEC/TERT1 OAT1, OCT2, and OAT3 Cells

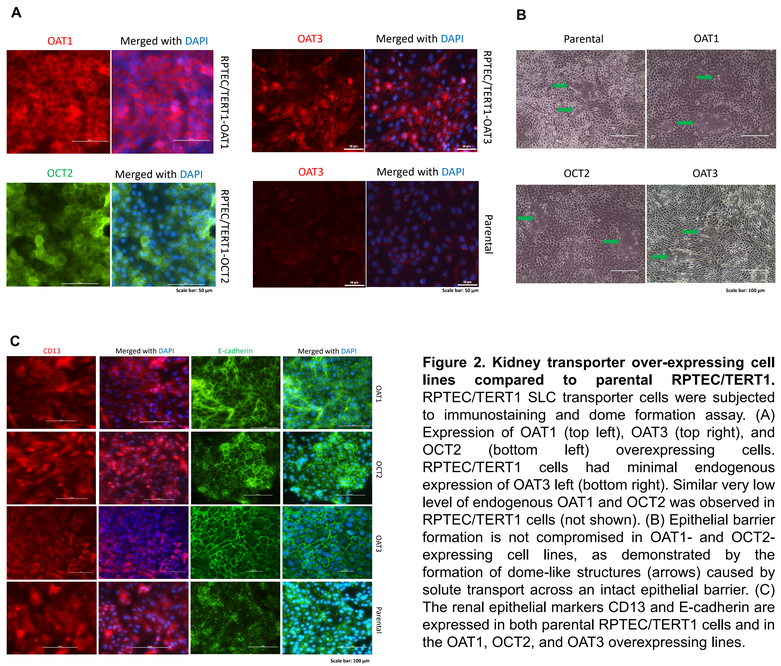
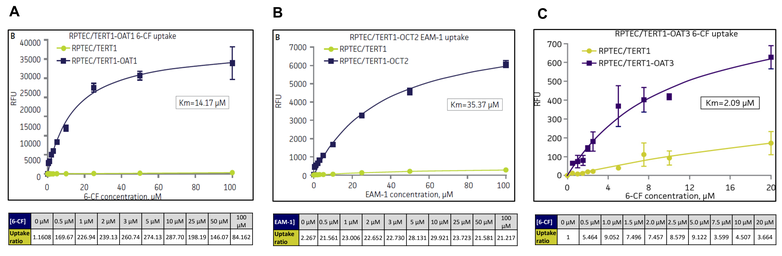


Figure 2. Kidney transporter over-expressing cell lines compared to parental RPTEC/TERT1. RPTEC/TERT1 SLC transporter cells were subjected to immunostaining and dome formation assay. (A) Expression of OAT1 (top left), OAT3 (top right), and OCT2 (bottom left) overexpressing cells. RPTEC/TERT1 cells had minimal endogenous expression of OAT3 left (bottom right). Similar very low level of endogenous OAT1 and OCT2 was observed in RPTEC/TERT1 cells (not shown). (B) Epithelial barrier formation is not compromised in OAT1- and OCT2-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 overexpressing lines.

III. Drug Uptake in RPTEC/TERT1 OAT1, OCT2, and OCT3 Cells



[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	3.664

Figure 3. Drug kinetic profiles of RPTEC/TERT1 OAT1, OCT2, and OAT3 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, and only very slightly 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 OAT3-expressing cells, and slightly in parental RPTEC/TERT1 cells (n=3), indicating that the observed transport is due to OAT3 expression (n=3). Small uptake observed in parental cells for EAM-1 and 6-CF may be due to remaining low endogenous levels of these transporters.

IV. Inhibition of Transport Activity in OAT1-, OCT2-, and OAT3-overexpressing RPTEC/TERT1 Cells

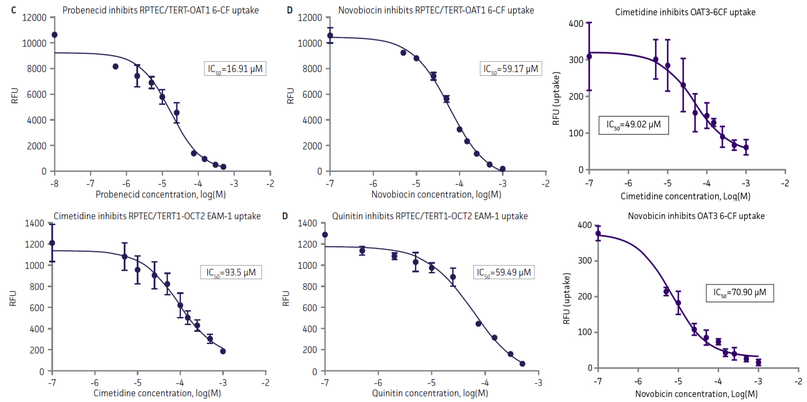


Figure 4. Transport inhibition kinetics of RPTEC/TERT1 OAT1-, OCT2-, and OAT3-expressing cell lines. OAT1-expressing cells were exposed to increasing concentrations of the known OAT1 inhibitors probenecid (top left) and novobiocin (top middle) to study their effect on 6-CF fluorescence substrate uptake. In a similar fashion, the effect of cimetidine (bottom left) and quinitin (bottom middle) on OCT-2-mediated EAM-1 uptake; and the effect of cimetidine (top right) and novobiocin (bottom right) on 6-CF uptake by OAT-3-expressing RPTEC/TERT1 cells was investigated. In each of the cases, a dose-dependent inhibition of transport activity was observed, indicating that OAT1, OCT2, and OAT3 have physiologically relevant transport activity when overexpressed in RPTEC/TERT1 cells.

V. Use of Transporter-expressing RPTEC/TERT1 Cells in Mechanistic Studies

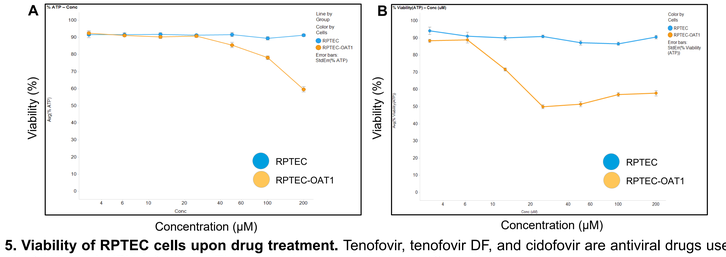


Figure 5. Viability of RPTEC cells upon drug treatment. Tenofovir, tenofovir DF, and cidofovir are antiviral drugs used in the treatment of HIV and HBV infections. They have known nephrotoxic effects that can in some instances be decreased by coadministration of other drugs (*e.g.*, cidofovir administered with probenecid). An example of how RPTEC/TERT1 cells overexpressing the transporters OAT1, OCT2, and OAT3 to study the mechanism of toxicity is demonstrated. Incubation of (A) tenofovir and (B) cidofovir with OAT1-overexpressing RPTEC/TERT1 cells (yellow trace) displayed a concentration-dependent loss of cell viability. This was in contrast to parental RPTEC1/TERT1 cells that showed no decrease in viability over a wide drug concentration range. These results demonstrate role of OAT1 in cytotoxicity for these drugs. Data was kindly provided by Merck & Co., Inc.

Summary

- RPTEC cells immortalized with TERT1 show similar marker expression (CD13, E-cadherin) and structural features (dome formation and transepithelial resistance) when compared to primary cells
- Overexpression of transporter proteins OAT1, OCT2, and OAT3 in the RPTEC/TERT1 cells generates an excellent model system for studying the function and physiological response of RPTEC cells
- RPTEC/TERT1-OAT1, -OCT2, and -OAT3 can be used in studying drug uptake, uptake inhibition, and mechanism of drug action (such as drug-mediated cytotoxicity)
- Overall, these studies demonstrate the power of immortalized TERT1 technology in generating relevant cell models for drug development and toxicity studies

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