Kidney SLC Transporter Cells – Reliable Tools for Assessing Renal Solute Passage and Drug Toxicity

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Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD.

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Agenda

- Renal transport
- Current renal models
- Generation of RPTEC renal uptake models
- Application data
- Summary
Renal transport proteins

- Play important roles in drug absorption, distribution, and elimination
- Can be divided into 2 classes:
  - The ATP-binding cassette (ABC) family, most are efflux transporters
  - The solute carrier (SLC) family, most are influx transporters, some are efflux and bidirectional
- Expression and activities are significant determinants for:
  - Drug disposition
  - Drug-drug interactions
  - Variability in drug response and toxicity
- Focus of today’s study:
  - Organic anion transporter protein 1 (OAT1)
  - Organic cation transporter protein 2 (OCT2)
  - Organic anion transporter protein 3 (OAT3)
Kidney cells – Role of OAT1/OCT2/OAT3

- Challenge: Expression of organic solute carrier transporters is lost in primary kidney cells

- Organic anion and cation transporters are vital in kidney metabolism
  - OAT1
  - OCT2
  - OAT3

- FDA guidance recommend evaluation of Oat/Oct transporter interactions:
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Cell lines and primary cell-derived models

Problems with primary kidney cell models:

Cell Lines:
- Problems with these lines:
  - Do not have the human kidney tissue origination
  - The cell line itself is a cancer line
- Therefore, the clinical predictability is greatly compromised

Primary Cells:
- Obtaining primary cultures is difficult
  - Homogeneous cultures retaining physiological functions are hard to obtain
- Primary RPTEC lose OAT1, OCT2, and OAT3 expression in culture
- Transiently expressing transporters in primary RPTEC show large variations between production lots
  - Makes the data hard to interpret

MDCK cells (ATCC® CCL-34™), image courtesy of Christopher Chin
hTERT immortalization technology

Regulation of telomere length in normal and cancer cells by telomerase

Expert Reviews in Molecular Medicine © 2002 Cambridge University Press
## Characteristics of various cell models

<table>
<thead>
<tr>
<th></th>
<th>Continuous (cancer) cell lines</th>
<th>Primary cells</th>
<th>hTERT-immortalized primary cells</th>
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<tbody>
<tr>
<td>Mimic <em>in vivo</em> characteristics</td>
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<tr>
<td>Proliferative capacity</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Experimental reproducibility</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Predictability in toxicological studies</td>
<td>+</td>
<td>+++</td>
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<tr>
<td>Genomic stability</td>
<td>Aneuploid</td>
<td>Diploid</td>
<td>Diploid/near diploid</td>
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<td>Supply</td>
<td>+++</td>
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<tr>
<td>Cost</td>
<td>+++</td>
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<td>++</td>
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<tr>
<td>Ease of use</td>
<td>+++</td>
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**hTERT-immortalized primary cells avoid the limitations of primary cells and continuous cell lines while reaping the benefits of both!**
Kidney models

Renal proximal tubule epithelial cells

- Primary renal proximal tubule epithelial cells
- hTERT-RPTEC – immortalized renal proximal tubule epithelial cells

Key characteristics:
- Uniform expression of E-cadherin and CD13 (aminopeptidase N)
- Formation of dome-like structures
- Stabilized transepithelial electrical resistance (TEER)
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Enhanced kidney cellular models

- RPTEC/TERT1
- RPTEC/TERT1 OAT1
- RPTEC/TERT1 OCT2
- RPTEC/TERT1 OAT3

Surviving RPTEC/TERT1 cells

Clonal selection, validation, and expansion

OAT1
OCT2
OAT3

Antibiotic selection

Characterized by RT-PCR, WB, sequencing (copy number verified)
Expression and localization of OAT1/OCT2/OAT3
Endogenous marker expression and dome formation

CD13  Merged with DAPI  E-cadherin  Merged with DAPI

Parental  OAT1

OCT2  OAT3

Scale bar: 100 µm

Scale bar: 100 µm
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Functionality – Drug uptake assay

UPTAKE ASSAY PROTOCOL

- Equal numbers of both parental and transporter cells were seeded into 96-well plate in triplicate for 24 hours
- Increasing concentration of 6-CF or EAM1 were added and incubated for 20 minutes at 37°C
- After wash with cold HBSS 4 times, cells were lysed and uptake intensity were measured
Functionality – Drug uptake inhibition assay

UPTAKE INHIBITION ASSAY PROTOCOL

- Equal numbers of both parental and transporter cells were seeded into 96-well plate in triplicate for 24 hours.
- Increasing concentration of inhibitors were added together with constant concentrations of the uptake substrate and incubated for 20 mins at 37°C.
- After wash with cold HBSS 4 times, cells were lysed and uptake intensity were measured.

6-CF uptake inhibition in OAT-1 expressing RPTEC

EAM-1 uptake inhibition in OCT-2 expressing RPTEC

6-CF uptake inhibition in OAT-3 expressing RPTEC

IC_{50}=59.17 μM

IC_{50}=93.5 μM

IC_{50}=70.90 μM
UPTAKE INHIBITION ASSAY PROTOCOL

- Aspirate growth media and wash once with warm 1X PBS; remove PBS and add 250 µL of cold inhibitors (prepared serum free DMEM, 0.5 µM) and incubate for 15 minutes
- Remove inhibitors and add 250 µL of radio-labeled TEA or metformin (prepared serum free DMEM, 4.5 µM) and incubate for 15 minutes
- Remove drug and wash 3 times with cold PBS; lyse the cells and count

Data kindly provided by:
Kevin Huang, Graduate Research Associate, Ohio State University, College of Pharmacy
Alice Gibson, Ph.D., Senior Research Specialist, Ohio State University, College of Pharmacy
Application for nephron toxicity studies

CELL VIABILITY ASSAY PROTOCOL

- About 35000 cells were seeded per well in triplicate in a 96-well plate and incubated overnight
- Cells were incubated with a series of compounds at various concentrations for 3 days
- Cell viability was determined using a cell viability assay per manufacturer’s instructions

Example 1: Tenofovir toxicity is OAT1 dependent

Example 2: Tenofovir DF toxicity is OAT1 independent

Example 3: Cidofovir toxicity is OAT1 dependent

Data kindly provided by: Merck & Co., Inc.
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Summary

- hTERT immortalized primary cells provide primary cell functionality with continuous cell line longevity
- We generated the clonal RPTEC/TERT1 renal uptake cell models by stably overexpressing OAT1, OCT2, and OAT3
- Expression was confirmed via genomic and expression data
- Clonal stable cells retain the original characteristics of RPTEC/TERT1.The performance of those stable cells was well-characterized
- Data from two outside collaborators indicating that the hTERT-immortalized primary RPTEC that express SLC transporters have multiple applications for toxicity testing.
- For more information visit

www.atcc.org/tox
Thank you and questions?

Coming soon!
Modeling Toxicity with Neural Progenitor Cell-derived Neurospheres
Presented by Brian Shapiro
February 24, 12:00 PM EST

Reproducibility and Physiological Relevance: The ATCC Toxicology Portfolio Webinar
Presented by Kevin Grady and Kevin Tyo
March 3, 12:00 PM EST

Society of Toxicology 61st Annual Meeting and ToxExpo
San Diego Convention Center, San Diego, California
March 28-30, Booth #1810

www.atcc.org/TOX