Genetically Modified Human Renal Proximal Tubule Epithelial Cells (RPTEC/TERT1) – A New Model for Drug Toxicity Studies

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Senior Scientist, ATCC
About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA

- World’s premiere biological materials resource and standards development organization

- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions

- Strong team of 400+ employees; over one third with advanced degrees
Agenda

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

Play important roles for drug:
- Absorption
- Distribution
- 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 at the basolateral and apical side of transporting epithelia are significant determinants for:
- Drug disposition
- Drug-drug interactions
- Variability in drug response and toxicity

Kidney epithelial cells recapitulate *in vivo* tubule formation, image courtesy of Moe Mahjoub
## Toxicologically important transport proteins

<table>
<thead>
<tr>
<th>Transporter/alias</th>
<th>Organs/cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP1B1/OATP-C, OATP2, LST-1</td>
<td>Hepatocytes (sinusoidal)</td>
</tr>
<tr>
<td>OATP1B3/OATP-8 (SLCO1B3)</td>
<td>Hepatocytes (sinusoidal)</td>
</tr>
<tr>
<td>OAT1 (SLC22A6)</td>
<td>Kidney proximal tubule, placenta</td>
</tr>
<tr>
<td>OAT3 (SLC22A8)</td>
<td>Kidney proximal tubule, choroid plexus, blood–brain barrier</td>
</tr>
<tr>
<td>OCT2 (SLC22A2)</td>
<td>Kidney proximal tubule, neurons</td>
</tr>
</tbody>
</table>

RPTEC/TERT1-OCT2 (ATCC® CRL-4031-OCT2™)
## Renal transport protein substrates and inhibitors

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Inhibitors</th>
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<tbody>
<tr>
<td>OAT1</td>
<td></td>
</tr>
<tr>
<td>- Cipro</td>
<td>- Probenecid</td>
</tr>
<tr>
<td>- Methotrexate</td>
<td>- Novobiocin</td>
</tr>
<tr>
<td>- Acyclovir</td>
<td>- Novobiocin</td>
</tr>
<tr>
<td>- Tenofovir</td>
<td>- Novobiocin</td>
</tr>
<tr>
<td>OAT3</td>
<td></td>
</tr>
<tr>
<td>- NSAIDS</td>
<td>- Probenecid</td>
</tr>
<tr>
<td>- Cefaclor</td>
<td>- Novobiocin</td>
</tr>
<tr>
<td>- Ceftizoxime</td>
<td>- Novobiocin</td>
</tr>
<tr>
<td>- Furosemide</td>
<td>- Novobiocin</td>
</tr>
<tr>
<td>- Bumetanide</td>
<td>- Novobiocin</td>
</tr>
<tr>
<td>OCT2</td>
<td></td>
</tr>
<tr>
<td>- Pindolol</td>
<td>- Cimetidine</td>
</tr>
<tr>
<td>- Amiloride</td>
<td>- Pilsialnide</td>
</tr>
<tr>
<td>- Oxalliplatin</td>
<td>- Etrizine</td>
</tr>
<tr>
<td>- Varenicline</td>
<td>- Testosterone</td>
</tr>
<tr>
<td></td>
<td>- Quinidine</td>
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</tbody>
</table>

**Focus on transporters in new regulatory documents**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>P-gp/MDR1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Multi</td>
</tr>
<tr>
<td>BCRP</td>
<td>+</td>
<td></td>
<td>+</td>
<td>Multi</td>
</tr>
<tr>
<td>BSEP</td>
<td></td>
<td>+</td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>+</td>
<td></td>
<td>+</td>
<td>Liver</td>
</tr>
<tr>
<td>OATP1B3</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Liver</td>
</tr>
<tr>
<td>OCT1</td>
<td></td>
<td></td>
<td>+</td>
<td>Liver</td>
</tr>
<tr>
<td>OAT1</td>
<td>+</td>
<td></td>
<td>+</td>
<td>Kidney</td>
</tr>
<tr>
<td>OAT3</td>
<td>+</td>
<td></td>
<td>+</td>
<td>Kidney</td>
</tr>
<tr>
<td>OCT2</td>
<td>+</td>
<td></td>
<td>+</td>
<td>Kidney</td>
</tr>
</tbody>
</table>

**New (draft) regulatory documents** published by FDA, EMA, and ITC recommended evaluate NME as substrate and drug interaction on the most important membrane transporters expressed in liver, intestine, and kidney
Agenda

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- Generation of RPTEC renal uptake models
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Cell line-based models

Current cell line-based models are available:
- MDCK (ATCC® CCL-34™)
- CHO-K1 (ATCC® CCL-61™)
- U-2 OS (ATCC® HTB-96™)
- Others

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 cell-derived models

Problems with primary kidney cell models:

- Obtaining primary cultures is difficult
  - The kidney comprises 15 cell types
  - The nephron comprises 20 cell types
  - 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
### Renal cell lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>ATCC® No.</th>
<th>Species of origin</th>
<th>Nephron segment of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLC-PK1</td>
<td>CL-101™</td>
<td>Yorkshire Pig</td>
<td>Proximal nephron</td>
</tr>
<tr>
<td>OK</td>
<td>CRL-1840™</td>
<td>North American Opossum</td>
<td>Proximal nephron</td>
</tr>
<tr>
<td>JTC-12</td>
<td>N/A</td>
<td>Monkey</td>
<td>Proximal nephron</td>
</tr>
<tr>
<td>MDCK</td>
<td>CCL-34™</td>
<td>Dog</td>
<td>Collecting duct</td>
</tr>
<tr>
<td>A6</td>
<td>CCL-102™</td>
<td><em>Xenopus laevis</em></td>
<td>Distal tubule</td>
</tr>
<tr>
<td>HK-2</td>
<td>CRL-2190™</td>
<td>Human</td>
<td>HPV16-transformed, Proximal/Distal?</td>
</tr>
<tr>
<td>Caki-1</td>
<td>HTB-46™</td>
<td>Human</td>
<td>Kidney carcinoma</td>
</tr>
<tr>
<td>HEK293/OAT1</td>
<td>CRL-11268G-1™</td>
<td>Human</td>
<td>Embryonic</td>
</tr>
</tbody>
</table>

None of the continuous renal epithelial cell lines fully recapitulate the functions of the parental cells *in vivo*
Pros and cons of different cell models for tissue-relevant functional studies

<table>
<thead>
<tr>
<th></th>
<th>Primary cells</th>
<th>hTERT immortalized</th>
<th>Oncogene, virally immortalized</th>
<th>Cancer cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mimic in vivo tissue phenotype</strong></td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><strong>Genotypic stability</strong></td>
<td>Diploid</td>
<td>Diploid / Near diploid</td>
<td>Near diploid / Aneuploid</td>
<td>Aneuploid</td>
</tr>
<tr>
<td><strong>Proliferative capacity</strong></td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Supply</strong></td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Inter-experimental reproducibility</strong></td>
<td>Low</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>High</td>
<td>Medium</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Ease-of-use</strong></td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>
hTERT-immortalized cells provide unique tools

hTERT-immortalized cells combine:

- The *in vivo* nature of primary cells
- The ability to be cultured continuously

hTERT-immortalized cells avoid the limitations of primary cells and continuous cell lines while still reaping their benefits

RPTEC/TERT1-OCT2 (ATCC® CRL-4031-OCT2™)
The parental cell

**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 and CD13 (aminopeptidase N)
- Dome-like structures
- Stabilized TEER
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Stable cell line generation

- OAT1, OCT2, and OAT3 delivery
- RPTEC/TERT1 cells
- Surviving RPTEC/TERT1 cells
- Clonal selection, Validation, and expansion
- RPTEC/TERT1-OAT1, OCT2, and OAT3 clonal cells

Antibiotic selection
RPTEC/TERT1-OAT1

RT-PCR

Western Blot

Sequencing: no mutation
OAT1 correctly localizes to the cell membrane in RPTEC/TERT1
RPTEC/TERT1-\textsc{OCT2}

A. RT-PCR

B. Western Blot

C. Sequencing: No mutation
OCT2 correctly localizes to cell membrane in RPTEC/TERT1
Growth characteristics of stably transfected RPTEC/TERT1

<table>
<thead>
<tr>
<th>Low density</th>
<th>RPTEC/TERT1</th>
<th>RPTEC/TERT1-OAT1</th>
<th>RPTEC/TERT1-OCT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>High density</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scale bar: 400 µm
RPTEC/TERT1 renal uptake key marker staining

CD13

Merged with DAPI

E-cadherin

Merged with DAPI

Scale bar: 100 µm
Dome formation

RPTEC/TERT1

RPTEC/TERT1-OAT1

RPTEC/TERT1-OCT2

Scale bar: 100 µm
Agenda

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RPTEC/TERT1-OAT1 drug kinetic profile

RPTEC/TERT1-OAT1 5-CF uptake

EC_{50}=89.21\mu M
Known OAT1 inhibitors block the RPTEC/TERT1-OAT1 5-CF uptake

Probenecid Inhibits RPTEC/TERT1 OAT1 5-CF uptake

Novobiocin Inhibits RPTEC/TERT1 OAT1 5-CF uptake

IC\textsubscript{50}=4.48\mu M

IC\textsubscript{50}=77.63\mu M
RPTEC/TERT1-OCT2 drug kinetic profile

RPTEC/TERT1-OCT2 uptake assay

![Graph showing uptake (RFU) vs ASP+ concentration (μM)]

- RPTEC/TERT1
- RPTEC/TERT1-OCT2

OCT2 EC<sub>50</sub> = 15.43 μM
Known OCT2 inhibitors block the RPTEC/TERT1-OCT2 Asp+ uptake

Cimetidine inhibits OCT2 Asp+ uptake

Quinitin inhibits OCT2 ASP+ uptake

IC50 = 138.9 μM

IC50 = 55.14 μM
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We generated clonal RPTEC/TERT1 renal uptake cell models by stably expressing OAT1 and OCT2 proteins

- Expression has been confirmed by:
  - PCR
  - Western blot
  - Immunocytochemistry

The clonal stable cells keep the original characteristics of the RPTEC/TERT1 cells

The performance of these stable cells are well characterized by:

- 5-CF uptake assays
- ASP uptake assays
- Inhibitor assays
Disclaimers

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