Best of Both Worlds – hTERT-immortalized Primary Cells

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ATCC Today

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA and an R&D & Services center in Gaithersburg, MD
- World wide brand name and quality recognition
- World’s premiere biological materials resource and standards development organization
  - 4,000 cell lines
  - 70,000 microbes
- ATCC collaborates with and supports the scientific community with industry-standard and innovative biological solutions
  - Growing portfolio of products and services
  - Sales and distribution in 140 countries, 12 International distributors
- Talented team of 475+ employees; > one third with advanced degrees
- Multiple accreditations including ISO 9001 and ISO 13485
Agenda

- hTERT-immortalized Primary Cells Portfolio
- Kidney transporter models
  - Current *in vitro* transporter cell models
  - Generation of RPTEC renal transporter models
  - Application data
ATCC classic and advanced cell models

- ATCC is the complete solution supplier
- From basic research through discovery and development to product testing
  - Continuous cell lines
  - Primary cells
  - hTERT immortalized primary cells
- Portfolio features
  - Reliability
  - Fully characterized cells
  - Optimized growth protocols
## Pros and cons of different cell models

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<thead>
<tr>
<th></th>
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<td>++++</td>
<td>+++</td>
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<td><strong>Genotypic stability</strong></td>
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<td>+</td>
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hTERT-immortalized cells combine:
- The *in vivo* nature of primary cells
- The ability to be continuously cultured

hTERT-immortalized cells avoid the limitations of primary cells while still reaping their benefits
ATCC portfolio – skin models

Primary Cells
- Keratinocytes
- Dermal Fibroblasts
- Melanocytes
- Dermal Microvascular Endothelial

hTERT Immortalized Primary Cells
- Keratinocytes
- Dermal Fibroblasts
- Dermal Microvascular Endothelial
- Melanocytes
ATCC keratinocytes cultured at ALI display similar architecture to skin *in vivo*
ATCC portfolio – airway models

Primary Cells
- Lung Fibroblasts
- Bronchial/Tracheal Epithelial Cells
- Small Airway Epithelial Cells
- Lung Smooth Muscle Cells
- Bronchial/Tracheal Smooth Muscle Cells

hTERT Immortalized Primary Cells
- Bronchial/Tracheal Epithelial Cells
- Small Airway Epithelial Cells
- Lung Fibroblasts
ATCC portfolio – urogenital models

Primary Cells
- Prostate Epithelial Cells
- Vaginal Epithelial Cells
- Uterine Fibroblast Cells
- Uterine Smooth Muscle Cells
- Bladder Epithelial (A/T/N) Cells
- Bladder Smooth Muscle Cells
- Bladder Fibroblast Cells

hTERT Immortalized Primary Cells
- Prostate Fibroblast Cells
- Prostate Cancer-associated Fibroblast Cells
- Prostate Epithelial Cells
- Endometrial Fibroblast Cells
A representative image of growth and staining of normal prostate epithelial cells in the presence of prostate normal-associated fibroblast (NAF) and prostate cancer-associated fibroblast (CAF). Data were then analyzed for percent change of growth of the experimental cells based on cell densities between the cells in the presence and absence of fibroblast cells.
ATCC portfolio – mesenchymal stem cells

Primary Stem Cells
- Adipose-derived Mesenchymal Stem Cells
- Subcutaneous Pre-adipocytes
- Bone Marrow-derived Mesenchymal Stem Cells
- Umbilical Cord-derived Mesenchymal Stem Cells

hTERT Immortalized Primary Cells
- Adipose-derived Mesenchymal Stem Cells
hTERT-immortalized and primary mesenchymal stem cells express equivalent markers

Flow cytometric analysis of surface marker expression determined ATCC hTERT-mesenchymal stem cells (MSCs) were equivalent to primary MSCs:

- Bone marrow (BM), adipose tissue (AT), umbilical cord (UC), and hTERT-derived MSCs were tested
- These data meet International Society for Cellular Therapy (ISCT) guidelines

<table>
<thead>
<tr>
<th>CD Marker</th>
<th>CD14 (%)</th>
<th>CD19 (%)</th>
<th>CD34 (%)</th>
<th>CD45 (%)</th>
<th>CD29 (%)</th>
<th>CD44 (%)</th>
<th>CD73 (%)</th>
<th>CD90 (%)</th>
<th>CD105 (%)</th>
<th>CD166 (%)</th>
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<tr>
<td>BM-MSC</td>
<td>0.26</td>
<td>0.07</td>
<td>2.91</td>
<td>0.15</td>
<td>100</td>
<td>99</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>94</td>
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<tr>
<td>AT-MSC</td>
<td>0.55</td>
<td>0.23</td>
<td>2.86</td>
<td>0.29</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>99</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>UC-MSC</td>
<td>0.52</td>
<td>0.79</td>
<td>1.50</td>
<td>0.47</td>
<td>100</td>
<td>90</td>
<td>95</td>
<td>96</td>
<td>94</td>
<td>95</td>
</tr>
<tr>
<td>hTERT-MSC</td>
<td>0.25</td>
<td>0.23</td>
<td>0.99</td>
<td>0.55</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>99</td>
<td>96</td>
</tr>
</tbody>
</table>
A co-culture system consisting of telomerase-immortalized endothelial cells and mesenchymal stem cells, providing an *in vitro* angiogenesis system that is more close to the *in vivo* situation

- A mix of TeloHAEC-GFP (ATCC® CRL-4054™) and ASC52telo (ATCC® SCRC-4000™) provided with an optimized medium formulation
Sunitinib blocks tubular structure growth in dose-dependent manner in the Angio-Ready™ System

TeloHAEC-GFP and hTERT-MSC cells premixed, thawed and seeded immediately into wells of a 96-well plate, and treated with different doses of sunitinib; fixed and stained with anti-αSMA antibody at day 8
ATCC portfolio – kidney models

**Primary Cells**
- Renal Proximal Tubule Epithelial Cells
- Renal Cortical Epithelial Cells
- Renal Mixed Epithelial Cells

**hTERT Immortalized Primary Cells**
- Renal Proximal Tubule Epithelial Cells
- Renal Proximal Tubule Epithelial Cells-OAT1
- Renal Proximal Tubule Epithelial Cells-OCT2
- Renal Proximal Tubule Epithelial Cells-OAT3 (coming soon)
Agenda

- hTERT-immortalized Primary Cells Portfolio
- Kidney transporter models
  - Current *in vitro* transporter cell models
  - Generation of RPTEC renal transporter models
  - Application data
The kidney is one of the major target organs for drug-induced toxicity

- Large functional reserve of the kidney
- Nephrotoxic effects become obvious only after regulatory approval

Nephrotoxic potential

- Often underestimated when new drugs are available
- Leads to clinical complications such as COX2 inhibitors

Renal proximal tubule (PT, blue box) is a major target for drug-induced toxicity due to its role in:

- Glomerular filtrate concentration
- Transport of drugs and organic compounds

Kidney drug toxicity and transporters

- 2 major transporters (ATP-binding Cassette transporters and **Solute Carrier transporters**)
- My talk today will only focus on SLC transporters
- New (draft) regulatory documents published by FDA, EMA, and ITC (2010 & 2012):
  
  “evaluate OAT1, OCT2, and OAT3 as major SLC targets for drug interaction testing expressed in the kidney”
Searching for the best *in vitro* models

To evaluate the drug transporter interactions, we need a good in vitro model with:

- High predictability
- Mature kidney PT origin
- Best mimics PT micro-environment

The central problem is the lack of good pre-clinical cell-based models
Searching for the best models: Primary cells

Con

Primary RPTEC lose OAT1, OCT2, and OAT3 expression in culture very quickly

Pro

Best represents the *in vivo* situation
### Searching for the best models: 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</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</td>
<td>CRL-11268™</td>
<td>Human</td>
<td>Embryonic, SV40T</td>
</tr>
</tbody>
</table>

None of the continuous renal epithelial cell lines have been fully characterized for recapitulating the functions of the PT cells *in vivo*
Searching for the best models: gene modification

Non-kidney or embryonic kidney transformed models (U2OS, CHO, etc...)

First-generation models

HEK293T17 overexpressing OAT1 (ATCC® CRL-11268-OAT1™)

Next-generation models

ATCC hTERT-immortalized RPTEC, stably transfected
Immortalized human adult proximal tubular cells: RPTEC/TERT1

RPTEC/TERT1 (ATCC® CRL-4031™)

- Derived from normal renal PT epithelium
- Immortalized using only hTERT

RPTEC/TERT1 exhibit:

- Uniform expression of E-cadherin and CD13 (aminopeptidase N)
- Dome*-like structures
- Stabilized TEER (Trans-Epithelial Electrical Resistance)

*Domes are multicellular, cystic structures, which have been described previously in epithelia cultured from various tissues that have a known transport or secretory function in vivo and in vitro
RPTEC/TERT1 cells, like its primary counterparts, lost OAT1, OCT2, and OAT3 expression in culture.
Characterization of RPTEC/TERT1-OAT1

Sequencing shows no mutation and 7 copies of OAT1 (vs 2 in the parental line)

A. RT-PCR

B. Western Blot

C. OAT1 Merged with DAPI

Scale bar: 100 µm
Characterization of RPTEC/TERT1-OCT2

A. RT-PCR

B. Western Blot

C. OCT2 Merged with DAPI

Sequencing shows no mutation and 5 copies of OCT2 (vs 2 in the parental line)
Preliminary characterization of RPTEC/TERT1-OAT3

RT-PCR

OAT3

Merged with DAPI

RPTEC/TERT1-OAT3

Parental

5 copies of OAT3 vs 2 parental
Dome formation – cells maintain original function

*Domes are multicellular, cystic structures, which have been described previously in epithelia cultured from various tissues that have a known transport or secretory function *in vivo* and *in vitro*. Domes are shown by the green arrows.
Key renal epithelial marker staining

CD13  Merged with DAPI  E-cadherin  Merged with DAPI

OAT1  OCT2  Parental

Scale bar: 100 µm
Key renal epithelial marker staining

CD13  Merged with DAPI  E-cadherin  Merged with DAPI

OA13

Parental

Scale bar: 100 µm
Agenda

- ATCC toxicology tools
- Kidney transporter models
  - Current \textit{in vitro} transporter cell models
  - Generation of RPTEC renal transporter models
  - Application data
Experimental design – brief assay protocol

**Uptake assay:**
- Equal numbers of both parental and transporter cells were seeded into 96-well plate in triplicate and placed in an incubator at 37°C, 5.0% CO₂.
- After 24 hours assays were performed.
- 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.

**Inhibition assay:**
- Equal numbers of both parental and transporter cells were seeded into 96-well plate in triplicate and placed in an incubator at 37°C, 5.0% CO₂.
- After 24 hours, assays were performed.
- 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.

These protocols apply to the following series of experiments.
OAT1, OCT2 and OAT3 displays high uptake sensitivity

<table>
<thead>
<tr>
<th>6-CF concentration</th>
<th>0 µM</th>
<th>0.5 µM</th>
<th>1 µM</th>
<th>2 µM</th>
<th>3 µM</th>
<th>5 µM</th>
<th>10 µM</th>
<th>25 µM</th>
<th>50 µM</th>
<th>100 µM</th>
</tr>
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<tbody>
<tr>
<td>Uptake ratio</td>
<td>1.16</td>
<td>169.67</td>
<td>226.94</td>
<td>239.13</td>
<td>260.74</td>
<td>274.13</td>
<td>287.70</td>
<td>198.19</td>
<td>146.07</td>
<td>84.16</td>
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<table>
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<tr>
<th>EAM-1 concentration</th>
<th>0 µM</th>
<th>0.5 µM</th>
<th>1 µM</th>
<th>2 µM</th>
<th>3 µM</th>
<th>5 µM</th>
<th>10 µM</th>
<th>25 µM</th>
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<tr>
<td>Uptake ratio</td>
<td>2.27</td>
<td>21.56</td>
<td>23.01</td>
<td>22.65</td>
<td>22.73</td>
<td>28.13</td>
<td>29.92</td>
<td>23.72</td>
<td>21.58</td>
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<th>1.5 µM</th>
<th>2.0 µM</th>
<th>2.5 µM</th>
<th>5.0 µM</th>
<th>7.5 µM</th>
<th>10 µM</th>
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<td>Uptake ratio</td>
<td>1.00</td>
<td>5.47</td>
<td>9.05</td>
<td>7.50</td>
<td>7.46</td>
<td>8.58</td>
<td>9.12</td>
<td>3.60</td>
<td>4.51</td>
<td>3.66</td>
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OAT1, OCT2 and OAT3 show accurate uptake specificity
Independent confirmation of the solute transport ability of RPTEC/TERT1-OCT2

Brief 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; after 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
Applications for nephron toxicity studies

The following data was kindly provided by Merck & Co., Inc.

Brief Protocol: Cell Viability Assay

- About 35000 cells were seeded per well in triplicate in a 96 well plate
  - Incubated overnight
  - Then incubated with a series of compounds at various concentrations as indicated for 3 days
- Cell viability was subsequently determined using CellTiter-Glo® Luminescent Cell Viability (ATP) Assay per Promega’s instructions
Tenofovir decreases cell viability in RPTEC/TERT1-OAT1 but not RPTEC/TERT1 cells

- Addition of probenecid, an inhibitor of OAT1, abolished the decrease in RPTEC/TERT1-OAT1 cell viability by tenofovir.

- Blocking of MRP4, the efflux transporter of tenofovir diphosphate, by ceefourin 2, further decreases cell viability presumably through increase of tenofovir diphosphate concentration in the cell.

Data kindly provided by: Merck & Co., Inc.
Cidofovir treatment decreases cell viability of RPTEC/TERT1-OAT1 but not RPTEC/TERT1 cells

Data kindly provided by: Merck & Co., Inc.
Modulation of cell viability with various test articles further indicates active transporter uptake

- RPTEC/TERT1-OAT1 cells but not the parent RPTEC/TERT1 cells demonstrate decreased cell viability following treatment of tenofovir, a substrate of OAT1 and 3.
- Blocking of MRP4, the efflux transporter of tenofovir diphosphate, by ceefourin 2, further decreases cell viability presumably through increase of tenofovir diphosphate concentration in the cell.
- Addition of probenecid, an inhibitor of OAT1, abolished the decrease in RPTEC/TERT1-OAT1 cell viability by tenofovir.
- Tenofovir, and cidofovir dose response curves show
  - All 3 compounds are toxic to RPTEC/TERT1-OAT1 cells
  - Tenofovir and cidofovir are not toxic to RPTEC/TERT1, since these compounds cannot enter the cells.

Data kindly provided by:
Merck & Co., Inc.
Utility of ATCC next-gen models in drug development

Screen with hTERT-immortalized SLC Renal Transport Models

Candidate Compound

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

Uptake or inhibition assay screening

If no change in transport: Further testing to determine transport mechanism

If positive or negative change in transport: Transport mechanism has been identified
**Summary**

ATCC RPTEC/TERT1 renal uptake cell models stably overexpress OAT1, OCT2, and OAT3

- Expression has been confirmed by:
  - PCR
  - Western blot
  - Immunocytochemistry
  - Copy number-verified

The clonal stable cells retain important characteristics of *in vivo* adult renal cells

The performance of these stable cells are well characterized by:

- 6-CF and EAM-1 uptake assays
- Inhibition assays
- Confirmed by TEA, Metformin and PAH uptake assays
- Tested against the well know renal toxic drugs
Resources

We have other hTERT cells – for a complete list download either:

- hTERT-immortalized Cell Culture Guide
- hTERT-immortalized Cells Brochure

For more information visit www.atcc.org/hTERT
Thank you

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