HTERT IMMORTALIZED CELL LINES – UNIQUE TOOLS FOR TISSUE-RELEVANT RESEARCH

Chengkang Zhang, Ph.D.
Senior Scientist
ASCB Vendor Showcase
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Outline

- History of cell culture, telomerase, and cell immortalization
- Create your own immortalized cell lines
- Overview of hTERT immortalized cell lines from ATCC
- Examples of hTERT immortalized cell lines
Primary human cell strains each have a characteristic replicative lifespan or "doubling potential", and that this lifespan is an intrinsic characteristic that can differ between strains.

- **Phase I** is the primary culture.
- **Phase II** represents subcultivated cells during the period of exponential replication.
- **Phase III** represents the period when cell replication ceases but metabolism continues. Cells may remain in this state for one year before death occurs.
Telomere and telomerase: The history


The Nobel Prize in Physiology or Medicine 2009
"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"

Elizabeth H. Blackburn
Carol W. Greider
Jack W. Szostak
Bypass replicative senescence by telomerase

Regulation of telomere length in normal and cancer cells by telomerase


http://www.senescence.info/telomeres_telomerase.html
Immortalization of normal human cells by hTERT

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Roads to cell immortalization


**Telomerase**
- hTERT
- HPV-16 E6
- Myc T58A

**p53/p21**
- SV40T
- HPV-16 E6

**p16/pRB**
- HPV-16 E7
- CDK4
- Bmi-1

**Other Methods**
- Feeder culture (3T3)
- Rho-associated kinase inhibitor (Y-27632)
- Physiological Oxygen (2-5%)
# Tools for cell immortalization

<table>
<thead>
<tr>
<th>Plasmids and Reagents</th>
<th>ATCC® No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>hTERT</td>
<td>MBA-141™</td>
</tr>
<tr>
<td>SV40-Baylor</td>
<td>VRMC-3™</td>
</tr>
<tr>
<td>HPV-16 E6/E7</td>
<td>CRL-2203™, 45113D™</td>
</tr>
<tr>
<td>CDK4</td>
<td>MGC-19704™, MGC-4678™, MGC-3719™</td>
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<tr>
<td>Bmi-1</td>
<td>81582D™, MGC-12685™</td>
</tr>
<tr>
<td>3T3 Feeder Cells</td>
<td>CCL-92™, 48-X™</td>
</tr>
<tr>
<td>ROCK Inhibitor Y-27632</td>
<td>ACS-3030™</td>
</tr>
</tbody>
</table>
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## hTERT immortalized cell lines from ATCC

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell Type</th>
<th>ATCC® No</th>
<th>Designations</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Mammary Epithelial</td>
<td>CRL-4010™</td>
<td>hTERT-HME1</td>
<td>Normal adult</td>
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<tr>
<td>Bone</td>
<td>Bone Cartilage Fibroblast</td>
<td>CRL-2846™, CRL-2847™</td>
<td>CHON-001, CHON-002</td>
<td>Normal fetal</td>
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<tr>
<td>Esophagus</td>
<td>Barrett's Esophageal Epithelial</td>
<td>CRL-4027™, CRL-4028™, CRL-4029™, CRL-4030™</td>
<td>CP-A, CP-B, CP-C, CP-D</td>
<td>Pre-malignant sample</td>
</tr>
<tr>
<td>Eye</td>
<td>Retinal Pigment Epithelial</td>
<td>CRL-4000™</td>
<td>hTERT-RPE1</td>
<td>Normal</td>
</tr>
<tr>
<td>Kidney</td>
<td>Angiomyolipoma</td>
<td>CRL-4004™</td>
<td>UMB1949</td>
<td>Angiomyolipoma</td>
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<tr>
<td></td>
<td>CRL-4008™</td>
<td></td>
<td>SV7tert PDGF tumor-1</td>
<td>Autocrine transformation and epigenetic changes</td>
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<tr>
<td></td>
<td>Proximal Tubule Epithelial</td>
<td>CRL-4031™</td>
<td>RPTEC/TERT1</td>
<td>Normal adult</td>
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<tr>
<td>Lung</td>
<td>Bronchial Epithelial</td>
<td>CRL-4011™</td>
<td>NuLi-1</td>
<td>Normal adult</td>
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<tr>
<td></td>
<td>CRL-4013™, CRL-4015™, CRL-4016™, CRL-4017™</td>
<td></td>
<td>CuFi-1, CuFi-4, CuFi-5, CuFi-6</td>
<td>Cystic Fibrosis</td>
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<tr>
<td></td>
<td>CRL-4051™</td>
<td></td>
<td>HBEC3-KT (coming soon)</td>
<td>Normal adult</td>
</tr>
<tr>
<td></td>
<td>Small Airway Epithelial</td>
<td>CRL-4050™</td>
<td>HSAEC1-KT (coming soon)</td>
<td>Normal adult</td>
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<tr>
<td>Pancreas</td>
<td>Pancreatic Duct</td>
<td>CRL-4023™</td>
<td>hTERT-HPNE</td>
<td>Normal adult</td>
</tr>
<tr>
<td>Skin</td>
<td>Foreskin Fibroblast</td>
<td>CRL-4001™</td>
<td>BJ-5ta</td>
<td>Normal neonatal</td>
</tr>
<tr>
<td>Keratinocyte</td>
<td>CRL-4048™</td>
<td></td>
<td>Ker-CT (just released)</td>
<td>Normal neonatal</td>
</tr>
<tr>
<td>Dermal Fibroblast</td>
<td>CRL-4005™</td>
<td></td>
<td>TelCOFS02MA (just released)</td>
<td>COFS</td>
</tr>
<tr>
<td>Uterus</td>
<td>Endometrium Stromal</td>
<td>CRL-4003™</td>
<td>T HESCs</td>
<td>Normal adult</td>
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<tr>
<td>Vascular</td>
<td>Microvascular Endothelial</td>
<td>CRL-4025™</td>
<td>TIME</td>
<td>Normal neonatal</td>
</tr>
<tr>
<td></td>
<td>Microvascular Endothelial</td>
<td>CRL-4045™</td>
<td>TIME-GFP (just released)</td>
<td>Stable GFP expression</td>
</tr>
<tr>
<td></td>
<td>Microvascular Endothelial</td>
<td>CRL-4049™</td>
<td>NFkB-TIME (coming soon)</td>
<td>NanoLuc reporter line</td>
</tr>
<tr>
<td>Aortic Endothelial</td>
<td>CRL-4052™</td>
<td></td>
<td>TeloHAEC (coming soon)</td>
<td>Normal adult</td>
</tr>
</tbody>
</table>
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RPTEC/TERT1 (CRL-4031™) – A new cell model

Limitations of existing *in vitro* renal cell cultures

**Primary Cells**

Obtaining primary cultures from the kidney is hampered by the fact that there are 15-20 cell types that comprise the kidney cortex and the nephron. Homogeneous cultures retaining physiological functions are hard to obtain.

**Immortal renal epithelial cell lines**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Derived from</th>
<th>Nephron Segment of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLC-PK1</td>
<td>Yorkshire Pig</td>
<td>Proximal nephron</td>
</tr>
<tr>
<td>OK</td>
<td>North American Opossum</td>
<td>Proximal nephron</td>
</tr>
<tr>
<td>JTC-12</td>
<td>Monkey</td>
<td>Proximal nephron</td>
</tr>
<tr>
<td>MDCK</td>
<td>Dog</td>
<td>Collecting duct</td>
</tr>
<tr>
<td>A6</td>
<td>Xenopus laevis</td>
<td>Distal tubule</td>
</tr>
<tr>
<td>HK-2</td>
<td>Human</td>
<td>HPV16-transformed, Proximal/ Distal?</td>
</tr>
<tr>
<td>Caki-1</td>
<td>Human</td>
<td>Kidney carcinoma</td>
</tr>
<tr>
<td>HEK293/OATs</td>
<td>Human</td>
<td>OATs over-expressing lines</td>
</tr>
</tbody>
</table>

None of the continuous renal epithelial cell lines fully express all the needed differentiated functions known from the ancestor cells *in vivo*. 
The RPTEC/TERT1 cells exhibit characteristic epithelial morphology only in the serum-free medium (♣) and express γ-Glutamyl Transferase (GGT), a marker protein located in the brush border of the renal proximal tubule epithelia.
The RPTEC/TERT1 cells propagate well and retain a normal male karyotype after extended culture in serum-free medium.
The RPTEC/TERT1 cells show uniform expression of E-cadherin and CD13(Aminopeptidase N), while primary RPTEC cells expression of these markers are highly variable.
Dome-like structures (arrows) form as water and solutes are transported across the cell layer and become trapped, the development of these structures is a good indicator of epithelial formation. Similarly, the formation of an intact epithelium can be demonstrated by stabilized Trans-Epithelial Electrical Resistance (TEER). RPTEC/TERT1 cells exhibit both dome-like structures and stabilized TEER, while the primary RPTEC cells do not possess either feature of intact epithelial formation.
RPTEC/TERT1 – Other interesting features


- **ABCC4 / MRP4**
- **ABCC2 / MRP2**
- **ABCB1 / MDR1 (P-gp)**
- **OATP4C1**
- **SLC47A1 / MATE1**
- **SLC22A12 / URAT1**

**T.** RPTEC/TERT1 (CRL-4031)  
**P.** Parental primary RPTEC  
**K.** Kidney tissue
**Ker-CT (CRL-4048™) – Immortalized keratinocyte**

Retain intact differentiation capability

- Ker-CT cell line was immortalized by human telomerase and CDK4 from neonatal foreskin keratinocyte culture (Deposited by Dr. Shay, UTSW)

TIME (CRL-4025™) – Good endothelial cell model

TERT Immortalized Microvascular Endothelial cells

- Immortalized from neonatal foreskin microvascular endothelial cells
- Proliferation to at least 200 population doublings
- Normal diploid karyotype
- Normal endothelial cell phenotype/function
  - Surface marker (PECAM-1/CD31, VEGFR2, Tie-2)
  - Ac-LDL uptake
  - Tubule formation on basement membrane gel
  - Support infection by endotheliotropic Kaposi’s sarcoma-associated herpes virus (KSHV/HHV-8)
  - Anoikis (apoptosis upon detachment from ECM)

TIME – Capable of forming vascular structure

Tubule formation on CellMatrix

TIME  ATCC® CRL-4025™
Media ATCC® PCS-100-030™
ATCC® PCS-110-040™ (BBE Kit)
ATCC® PCS-110-041™ (VEGF Kit)
CellMatrix ATCC® ACS-3035™

Tubule formation on co-culture

Co-cultured with BJ Cells
Day 14, CD31

vWF  CD31
Genetic engineered cell lines derived from TIME

TIME-GFP (ATCC® CRL-4045™)

- Derived by transfecting TIME (ATCC® CRL-4025™) cells with linearized pWE2-EmGFP plasmid
- Clonal cell line selected based its stably expression of GFP driven by CMV promoter
- Diploid cell line of male origin with a chromosome number of 46
- Positive for endothelial cell markers as the parental TIME cell line (CD31, AcLDL uptake, VEGFR-2, Tie-2)
- Tested for at least 15 population doublings after recovery from cryopreservation
- Tubule formation on CellMatrix

Poster# B1332, Monday, Dec 16th, 1:30-3:00 PM
Characteristics of TIME-GFP cell line

![Graph showing accumulative PDL vs days in culture for VEGF medium and BBE medium.](image1)

- **Phase**: Image showing phase contrast of TIME-GFP cells.
- **AcLDL**: Image showing red fluorescence of AcLDL uptake.
- **GFP**: Image showing green fluorescence of GFP expression.
- **VE-Cad**: Image showing red fluorescence of VE-Cadherin expression.

The graphs illustrate the proliferation and differentiation capacities of TIME-GFP cells under different media conditions.
GFP expression facilitates real-time analysis

The GFP-expressing cells migrate and coalesce into networks of vessel-like structure within 10 hours after being plated onto basement membrane gel (CellMatrix, ATCC® ACS-3035™). The stable expression of GFP in these cells enables detection and analysis of the fragile endothelial structures to occur without post-assay fixation and/or staining.
Genetic engineered cell lines derived from TIME

**NFKB-TIME (ATCC® CRL-4049™)**

- Derived by transfecting TIME (ATCC® CRL-4025™) cells with linearized pNL3.2-Nluc/NF-kB-RE/Hygro plasmid
- Clonal cell line selected based its high response to TNFα
- Normal diploid karyotype
- Positive for parental cell characteristics
  - TIME cell markers (CD31, AcLDL uptake)
  - Endothelial cell functions
- Tested for at least 15 population doublings after recovery from cryopreservation

https://www.promega.com/products/pm/nanoluc/
NFkB-TIME (CRL-4049™) reporter cell line

NFkB-TIME (ATCC® CRL-4049™) expresses NanoLuc® luciferase regulated by multiple copies of the NFkB response element. When the cells are exposed to inflammatory cytokine such as TNFα, activation of the NFkB signaling pathway results in increased NanoLuc® luciferase activity. The high sensitivity, excellent signal/background ratio and simple single-addition assay makes this reporter cell line an ideal replacement for the cumbersome and highly variable CD54/ICAM-1 activation assays.
Use of NanoLuc® increases assay sensitivity

Variable number of NFKB-TIME (ATCC® CRL-4049™) cells were seeded into 96-well plate and incubated for 24 hours in culture medium. The cells were then exposed to 100 ng/mL TNFα for 3 hours to activate the NFkB signaling pathway. Comparable fold of induction (FOI) of luminescence was observed within a wide range of cell seeding densities. Less than 100 cells/well produced significant activation of the reporter gene expression.
# hTERT immortalized cells provide unique tools

<table>
<thead>
<tr>
<th></th>
<th>Primary cells</th>
<th>hTERT immortalized</th>
<th>Oncogene, viral 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>

**Pros and cons of different cell models for tissue-relevant functional studies**

hTERT immortalized cells combine the *in vitro* nature of primary cells and the ability to be cultured continuously, avoiding the limitations of both types while still reaping their benefits.