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

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Senior Scientist, ATCC
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Who we are

• ATCC serves and supports the scientific community with industry-standard products and innovative solutions

• World’s leading biological resource center and provider of biological standards

• Broad range of biological materials
  – Microorganisms
  – Cell lines
  – Derivatives
  – Bioproducts

• Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
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
Inconvenience of primary cell culture
The Hayflick Limit

Who: Leonard Hayflick
When: 1965
Methods: Normal diploid cells were serially passaged in culture until they stopped dividing
Institution: Wistar Institute
Where: Philadelphia, PA, U.S.A

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

**1938/1941**
The ends of chromosomes had to be capped by a special structure termed “telomere”
H. Muller/B. McClintock

**1972**
"End replication problem"
J. Watson

**1978-1982**
Sequence of Tetrahymena telomere TTGGGG
E. Blackburn, J Szostak

**1985**
Discovering of Telomerase
C Greider, E. Blackburn

**1990**
Telomere hypothesis of cell senescence
- yeast
J Szostak
- human cells/cancer

**1994**
Development of the PCR-based “TRAP assay”

**1995**
Cloning of hTR

**1995/1997**
Characterization of TRF1/TRF2
Cloning of hTERT

**1997**
Generation of the "telomerase knockout mouse"

**1999/2000...**
Telomerase/telomere dysfunctions and cancer

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**The Nobel Prize in Physiology or Medicine 2009**

"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"

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Bypass replicative senescence by telomerase

Regulation of telomere length in normal and cancer cells by telomerase

Expert Reviews in Molecular Medicine ©2002 Cambridge University Press


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

Retinal Pigment Epithelial Cell  
CRL-4000™  
hTERT-RPE1

Foreskin Fibroblast  
CRL-4001™  
BJ-5ta

pGRN145, plasmid in E. coli
ATCC® MBA-141

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- Examples of hTERT immortalized cell lines
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>
</tr>
<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>
</tr>
<tr>
<td></td>
<td>CRL-4008™</td>
<td>SV7tert PDGF tumor-1</td>
<td>Autocrine transformation and epigenetic changes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>Bronchial Epithelial</td>
<td>CRL-4011™</td>
<td>NuLi-1</td>
<td>Normal adult</td>
</tr>
<tr>
<td></td>
<td>CRL-4013™, CRL-4015™, CRL-4016™, CRL-4017™</td>
<td>CuFi-1, CuFi-4, CuFi-5, CuFi-6</td>
<td>Cystic Fibrosis</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>CRL-4051™</td>
<td>HBECS3-KT (coming soon)</td>
<td>Normal adult</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td>Lung</td>
<td>Bronchial Epithelial</td>
<td>CRL-4050™</td>
<td>HSAEC1-KT (coming soon)</td>
<td>Normal adult</td>
</tr>
<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></td>
<td>Keratinocyte</td>
<td>CRL-4048™</td>
<td>Ker-CT (just released)</td>
<td>Normal neonatal</td>
</tr>
<tr>
<td></td>
<td>Dermal Fibroblast</td>
<td>CRL-4005™</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>
</tr>
<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 (just released)</td>
<td>NanoLuc reporter line</td>
</tr>
<tr>
<td></td>
<td>Aortic Endothelial</td>
<td>CRL-4052™</td>
<td>TeloHAEC (coming soon)</td>
<td>Normal adult</td>
</tr>
<tr>
<td>Adipose</td>
<td>Mesenchymal Stem Cell</td>
<td>SCRC-4000™</td>
<td>ASC52tel0 (just released)</td>
<td>Normal Adult</td>
</tr>
</tbody>
</table>
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hTERT Immortalized Endothelial Cell Lines – Good endothelial cell models

- Over-expression of ectopic telomerase can immortalize endothelial cells isolated from diverse tissues sources, e.g., umbilical cord vein, dermis of juvenile foreskin, aorta, etc.
  - Extended lifespan with normal diploid karyotype
  - Normal endothelial cell phenotype/function
    - Surface markers and receptors (PECAM-1/CD31, VEGFR2, Tie-2)
    - Ac-LDL uptake (LDL receptor functional assay)
    - Neoangiogenesis – Tubule formation on basement membrane gel

- ATCC’s hTERT-immortalized endothelial cells collection

<table>
<thead>
<tr>
<th>ATCC® Cat. No.</th>
<th>Cell Line</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRL-4052™</td>
<td>TeloHAEC</td>
<td>Normal adult aortic endothelial cells <em>(coming soon)</em></td>
</tr>
<tr>
<td>CRL-4025™</td>
<td>TIME</td>
<td>Foreskin microvascular endothelial cells</td>
</tr>
<tr>
<td>CRL-4045™</td>
<td>TIME-GFP</td>
<td>Foreskin microvascular endothelial cells with constitutive expression of EmGFP®</td>
</tr>
<tr>
<td>CRL-4049™</td>
<td>NFkB-TIME</td>
<td>Foreskin microvascular endothelial cells with NanoLuc® reporter expression under the control of NFkB response elements</td>
</tr>
</tbody>
</table>

NanoLuc® is a trademark of Promega, and EmGFP® is a trademark of Life Technologies.
TeloHAEC – immortalized aortic endothelial cell line

**Graph:**
- **Primary HAEC**
- **TeloHAEC**

**Karyotype Sample:**
- Normal Diploid Karyotype

**TeloHAEC**
- ATCC® CRL-4052™
- ATCC® PCS-100-030
- ATCC® PCS-110-040 (BBE Kit)
- ATCC® PCS-110-041 (VEGF Kit)

**Media**
- ATCC® ACS-3035

**Cell Basement Membrane Gel**
- ATCC® ACS-3035

**Tubule formation on Basement Membrane Gel**

**hTERT transduction**
TeloHAEC – consistent functionality over time

A. Upregulation of CD54/ICAM-1

B. Upregulation of CD62E/E-Selectin

C. Upregulation of CD106/VCAM-1

D. VEGF-stimulated cell growth
TeloHAEC – phenotypic angiogenesis assay

Long-term tubule formation in co-culture of endothelial cells and fibroblasts

Endothelial cells co-cultured with mesenchymal cells produce stable tubular structure that appears more representative of capillary formation in vivo.

TeloHAEC cells are co-cultured with BJ fibroblast for 14 days, and stained with anti-CD31 to reveal tubular structure.
TIME – immortalized microvascular endothelial cells

Tubule formation on Gel

Tubule formation on co-culture

**TIME**  ATCC® CRL-4025™

**Media**  ATCC® PCS-100-030

ATCC® PCS-110-040 (BBE Kit)

ATCC® PCS-110-041 (VEGF Kit)

Cell Basement Membrane Gel

Day 14, CD31

vWF

CD31
Genetic engineered cell lines derived from hTERT-immortalized endothelial cells

**TIME-GFP (ATCC® CRL-4045™)**
- Derived by transfecting TIME (ATCC® CRL-4025™) cells with linearized pWE2-EmGFP plasmid
- Clonal cell line selected based on its stable expression of EmGFP® driven by CMV promoter

**NFκB-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 on its high expression of NanoLuc® reporter in response to TNFα

**Both TIME-GFP and NFκB-TIME are:**
- Diploid cell line with a chromosome number of 46
- Positive for endothelial cell markers as the parental TIME cells (CD31, AcLDL uptake, VEGFR-2, Tie-2)
- Tubule formation on Gel
- Tested for at least 15 population doublings after recovery from cryopreservation
TIME-GFP – consistent endothelial phenotype over extended culture

![Graph showing cumulative PDL over days in culture with VEGF medium and BBE medium](image)

![Phase contrast image](image)

![AcLDL staining](image)

![FACS analysis](image)

![GFP and VE-Cad immunostaining](image)
TIME-GFP – GFP expression facilitates real-time analysis

The GFP-expressing cells migrate and coalesce into networks of vessel-like structures within 10 hours after being plated onto Cell Basement Membrane Gel (ATCC® ACS-3035™). The stable expression of GFP in these cells enables the detection and analysis of the fragile endothelial structures to occur without post-assay fixation and/or staining.
NFkB-TIME – NanoLuc® reporter expression correlates with endogenous marker

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.
NFkB-TIME – use of NanoLuc® reporter increases assay sensitivity

A variable number of NFκB-TIME (ATCC® CRL-4049™) cells were seeded into a 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.
Ker-CT (CRL-4048™) – immortalized keratinocytes that 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)

RPTEC/TERT1 (CRL-4031™) — a new RPTEC cell line overcomes limitations of existing renal cell models

Primary Cells
Obtaining primary cultures from the kidney is hampered by the fact that there are 15-20 cell types in 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 propagate well and retain a normal male karyotype after extended culture in serum-free medium. After recovery from cryopreservation, RPTEC/TERT1 at P21 maintains an extended lifespan and stable karyotype.
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 (indicated by the arrows) form as water and solutes are transported across the cell layer and become trapped underneath; the development of these structures is a good indicator of intact epithelium 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
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>
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<tr>
<td><strong>Supply</strong></td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<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>
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<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.
Thank you!

Register for more webinars in the ATCC “Excellence in Research” webinar series at www.atcc.org/webinars.

April 24, 2014
10:00 AM, 3:00 PM EST
Dr. Fang Tian will highlight cell lines that can be used to address recently identified genomic and clinical features of breast cancer subtypes.

May 8, 2014
10:00 AM, 3:00 PM EST
Liz Kerrigan will discuss the importance of molecular standards, and how their use can contribute to improvements in assay reproducibility and reliability.

June 5, 2014
10:00 AM, 3:00 PM EST
Dr. Doug Storts and Dr. Yvonne Reid will discuss the recent advances in STR profiling technologies and how the Standard STR protocol is transforming scientific practices.

Thank you for joining today!
Please send additional questions to tech@atcc.org